I U C L I D

Data Set

Existing Chemical	:	ID: 2386-87-0
CAS No.	:	2386-87-0
EINECS Name	:	7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate
EC No.	:	219-207-4
Molecular Formula	:	C14H20O4
Structural Formula	:	(C6H9O)COOCH2C6H9O

Producer related part

Company	:	Dow Chemical, TERC
Creation date	:	17.08.2004

Substance related part

Company	:	Dow Chemical, TERC
Creation date	:	17.08.2004
Status	:	
Memo	:	
Printing date	:	08.12.2005
Revision date	:	
Date of last update	:	08.12.2005
Number of pages	:	73
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	
Name	:	Dow Chemical Company
Contact person	:	
Date	:	
Street	:	
Town	:	48674 Midland, MI
Country	:	United States
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Email	:	
Homepage	:	
Source	:	Dow Chemical Company The Dow Chemical Company Midland, Michigan

28.08.2003

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type	:	
Substance type	:	organic
Physical status	:	liquid
Purity	:	= 82 - 89 % w/w
Colour	:	
Odour	:	
Source	:	Dow Chemical Company The Dow Chemical Company Midland, Michigan
Reliability	:	(2) valid with restrictions

28.08.2003

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

7-Oxabicyclo[4.1.0]heptane-3-carboxylic acid, 7-oxabicyclo[4.1.0]hept-3-ylmethyl ester

Source : Dow Chemical Company
The Dow Chemical Company Midland, Michigan

08.05.1998

Cycloaliphatic Epoxy Resin ERL-4221

Source : Dow Chemical Company
The Dow Chemical Company Midland, Michigan

28.08.2003

ERL-4221

Source : Dow Chemical Company
The Dow Chemical Company Midland, Michigan

28.08.2003

1.3 IMPURITIES

Purity :
CAS-No :
EC-No :
EINECS-Name : oligomer of ERL-4221
Molecular formula :
Value : = 8 - 13 % w/w

Reliability : (1) valid without restriction
22.08.2003 (1)

Purity :
CAS-No :
EC-No :
EINECS-Name : monoepoxide of ERL-4221
Molecular formula :
Value : = 0 - 5 % w/w

Reliability : (1) valid without restriction
22.08.2003 (1)

Purity :
CAS-No : 2611-00-9
EC-No :
EINECS-Name : 3-cyclohexene-1-carboxylic acid, 3-cyclohexen-1-ylmethyl ester
Molecular formula :
Value : <= .3 % w/w

Reliability : (1) valid without restriction
12.12.2003 (1)

1.4 ADDITIVES

1.5 TOTAL QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use : industrial
Category : other: coating
28.08.2003

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

1.13 REVIEWS

2.1 MELTING POINT

Value	:	ca. -30 - -35 °C
Sublimation	:	
Method	:	OECD Guide-line 102 "Melting Point/Melting Range"
Year	:	2000
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	A differential scanning calorimeter was used. Approximately 14mg test material was cooled to -120C, held at a steady temperature for two minutes and then scanned upwards to 25C at 10K/min. The test was repeated with the sample cooled to -90C. Due to the effects observed, approximately 5 ml sample of test material was cooled in an open test tube by placing it in a wider tube located in a Drikold bath. Readings were taken of the temperature and visual observations made of the state of the sample. A glass rod was used to help assess fluidity.
Result	:	Using differential scanning calorimetry, there was no evidence of exothermic crystallization nor endothermic melting. A step change at approximately -60C in both directions, however, indicated a glass transition, a property associated with non-crystalline solids. The same result was observed in the repeat test. Using the Drikold bath, the sample became more viscous. At a temperature of approximately -30C the test material was essentially liquid. At -35C, the sample could not be stirred and it was therefore regarded as solid. Cooling was continued to -49C, at which temperature it was hard solid. It was then allowed to warm up smoothly. Above -35C the sample softened and above -30C it was sufficiently mobile to pour. It was therefore regarded as liquid and the viscosity decreased steadily as the temperature rose further. A plot of the temperature vs time showed that during both cooling and warming there was no isothermal stage that would indicate either crystallization or true melting, confirming the DSC observation.
Reliability	:	(1) valid without restriction 1a: GLP guideline study

23.07.2003 (2)

2.2 BOILING POINT

Value	:	> 300 °C at 1013 hPa
Decomposition	:	yes
Method	:	OECD Guide-line 103 "Boiling Point/boiling Range"
Year	:	2000
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	The test material was too viscous to determine the boiling point by ebulliometry. Therefore differential scanning calorimetry was used instead. A sample of ca 7 mg was heated at 10K/min from 30 to 450C in a nitrogen purge atmosphere. A second sample of ca 5 mg mass was heated similarly to 380C.
Result	:	Irregular exothermic behavior was observed above ca 300C, indicating decomposition in the first sample. The same result was obtained in the

second sample. Both crucibles contained a black residue on opening, confirming that decomposition had occurred.

Reliability : (1) valid without restriction
1a: GLP guideline study

23.07.2003 (3)

2.3 DENSITY

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : = .00002 hPa at 25 °C
Decomposition :
Method : OECD Guide-line 104 "Vapour Pressure Curve"
Year : 2000
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : The test method was effusion manometry. Temperatures used were 68.1, 82.1 and 110.9C. Data was extrapolated to 25C.
Result : At temperatures of 68.1, 82.1 and 110.9C the vapor pressure was 0.194, 0.779 and 6.35 Pa, respectively.

By extrapolation, the vapor pressure at 25C is 0.002 Pa or 0.00002 hPa.
Reliability : (1) valid without restriction
1a: GLP guideline study

05.09.2003 (4)

2.5 PARTITION COEFFICIENT

Partition coefficient :
Log pow : = 1.34 at 20 °C
pH value :
Method : OECD Guide-line 107 "Partition Coefficient (n-octanol/water), Flask-shaking Method"
Year : 2000
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : In a preliminary test, a solution of 1360 mg/L test material in water saturated n-octanol was prepared, mixed with an equal volume of n-octanol saturated water, rotated approximately 100 times within a 5 minute period and then left to settle. Two separate determinations were then made. Firstly the n-octanol and aqueous phases were transferred into separate clean glass tubes using pasteur pipettes. Samples were given a x10 dilution with toluene and analyzed by gas chromatography according to the method described in Appendix 1. These uncentrifuged samples gave an estimation without separation by centrifugation, which is required in the definitive test. Therefore the remainder of the separated n-octanol and water phases were centrifuged at 3200 ppm for 30 minutesthen aliquots diluted and analyzed as previously.

For the definitive study, the shake flask method was used. Initially, a nominal stock solution concentration of 1000 mg/L test material in water

saturated n-octanol was prepared for the definitive test. Duplicate samples were prepared in n-octanol saturated water:stock solution ratios of 1:2, 1:1 and 2:1. The test temperature was 20+/-1C.

Result : The average log Pow was 1.34. In 6 different measurements, the log Pow ranged from 1.32 to 1.36.

Attached document :

TABLE 1

ANALYTICAL RESULTS - PRELIMINARY TEST

	Nominal concn of Cycloaliphatic Epoxide Resin ERL-4221 in n-octanol stock ($mg\ l^{-1}$)	Measured concn of Cycloaliphatic Epoxide Resin ERL-4221 in n-octanol stock ($mg\ l^{-1}$)	Measured concn of Cycloaliphatic Epoxide Resin ERL-4221 in n-octanol ($mg\ l^{-1}$)	Measured concn of Cycloaliphatic Epoxide Resin ERL-4221 in deionised water ($mg\ l^{-1}$)
Uncentrifuged	1360	1200	1140	89
Centrifuged	1360	1290	1220	37

All results quoted to 3 significant figures

TABLE 2

PARTITION COEFFICIENT RESULTS - PRELIMINARY TEST

	Nominal concn of Cycloaliphatic Epoxide Resin ERL-4221 in n-octanol ($mg\ l^{-1}$)	n-octanol-water partition coefficient (P_{ow})	$Log_{10}P_{ow}$
Uncentrifuged	1360	12.8	1.11
Centrifuged	1360	33.0	1.52

All results quoted to 3 significant figures

TABLE 3

ANALYTICAL RESULTS - DEFINITIVE TEST

Vessel number	pH of aqueous phase	Nominal concn of Cycloaliphatic Epoxide Resin ERL-4221 in n-octanol ($mg\ l^{-1}$)	Measured concn of Cycloaliphatic Epoxide Resin ERL-4221 in n-octanol ($mg\ l^{-1}$)	Measured concn of Cycloaliphatic Epoxide Resin ERL-4221 in deionised water ($mg\ l^{-1}$)
1	7	1000	917	41.1
2	7	1000	858	41.3
3	7	1000	889	40.4
4	7	1000	932	40.8
5	7	1000	794	38.4
6	7	1000	835	38.6

Analytical measurement of test substance quoted to 3 significant figures

TABLE 4

PARTITION COEFFICIENT RESULTS - DEFINITIVE TEST

Vessel number	Nominal concn of Cycloaliphatic Epoxide Resin ERL-4221 in n-octanol ($mg\ l^{-1}$)	n-octanol-water partition coefficient (P_{ow})	$Log_{10}P_{ow}$	Mean of n-octanol water partition coefficient	Standard deviation of n-octanol water partition coefficient	Mean $Log_{10}P_{ow}$
1	1000	22.3	1.35	21.7	0.858	1.34
2	1000	20.8	1.32			
3	1000	22.0	1.34			
4	1000	22.8	1.36			
5	1000	20.7	1.32			
6	1000	21.6	1.33			

TABLE 5

TEMPERATURE MEASUREMENTS - DEFINITIVE TEST

Date	Room temperature		Waterbath temperature	
	Time	Temperature °C	Time	Temperature °C
22.3.00	09:10	20.0	09:30	20.6
22.3.00	10:10	20.0	10:30	20.2
22.3.00	11:10	20.3	11:15	20.2[b]
22.3.00	12:10	20.2	12:15	20.1
22.3.00	13:10	20.0	13:15	20.1[b]
22.3.00	14:10	20.1	-	-

Note

a Tubes placed in waterbath
b Tubes removed from waterbath

APPENDIX 1

DETERMINATION OF CYCLOALIPHATIC EPOXIDE RESIN ERL-4221 IN WATER/n-OCTANOL SAMPLES

Aqueous and octanol samples of Cycloaliphatic Epoxide Resin ERL-4221 were extracted or diluted respectively with toluene and analysed by gas chromatography using a flame ionisation detector. The samples were quantified against standards of test substance in toluene, prepared from an acetone or toluene stock.

GC Conditions

Column: 25 m x 0.32 mm id silica
Column stationary phase: CP-Sil 8CB
Column temperature:

	Start temp °C	Ramp rate °C min^{-1}	Final temp °C	Hold time min
initial	60	-	60	2.0
prgm 1	60	20	220	7.0

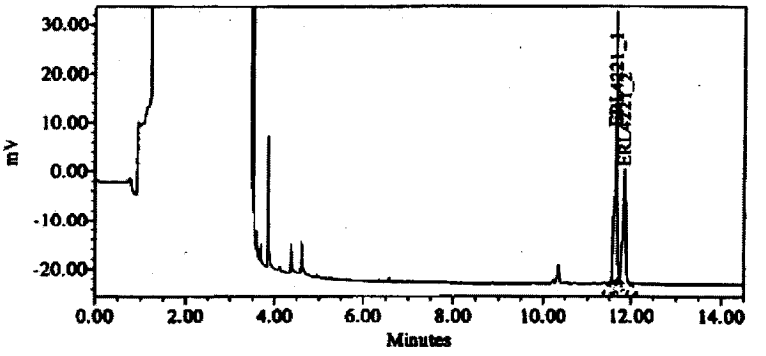
Injection port temperature: 250°C
Injection volume: 2 µl
Carrier gas flow rate: helium @ 2.5 ml min^{-1}
Detector: flame ionisation
Detector gases: nitrogen make up @ 35 ml min^{-1}
air @ 300 ml min^{-1}
hydrogen @ 25 ml min^{-1}
Detector temperature: 300°C
Detector range: 12

Using the above conditions, two peaks were obtained for Cycloaliphatic Epoxide Resin ERL-4221 with retention times of approximately 11.6 and 11.9 minutes. A 25 mg l^{-1} standard of Cycloaliphatic Epoxide Resin ERL-4221 in toluene produced approximately a total peak area (sum of two peaks) of 2.9×10^5 µV s using a Millennium^{32} (version 3.05.01) chromatographic data system.

APPENDIX 1 FIGURE 1

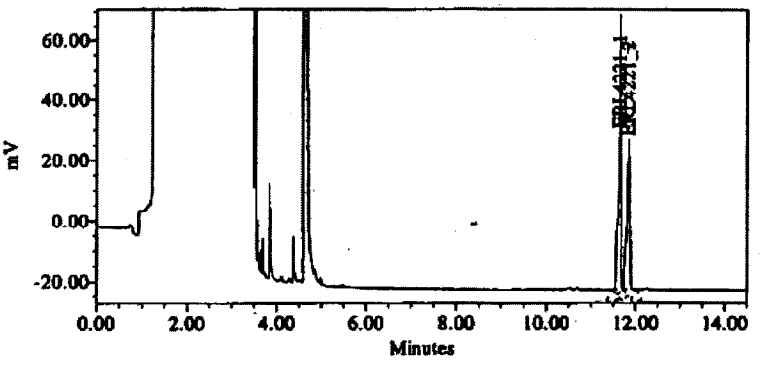
TYPICAL CHROMATOGRAMS

(A) 25 mg l^{-1} standard of Cycloaliphatic Epoxide Resin ERL-4221 in toluene



Unique_Number 22Mar00_19 Injection 2 SampleName S25 Date Acquired 23/03/00 01:52:24

(B) 1000 mg l^{-1} nominal concentration of Cycloaliphatic Epoxide Resin ERL-4221, water phase from tube 1 extracted 1:1 into toluene



Unique_Number 22Mar00_20 Injection 1 SampleName (26) tube 1 water 1:1ext Date Acquired 23/03/00 02:14:07

Reliability : (1) valid without restriction
1a: GLP guideline study

23.07.2003 (5)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	
Value	:	= 13850 mg/l at 20 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	very soluble (> 10000 mg/L)
Stable	:	
Deg. product	:	
Method	:	OECD Guide-line 105
Year	:	2000
GLP	:	yes

Test substance : as prescribed by 1.1 - 1.4

Method : In a preliminary study, increasing amounts of deionized water was added to 0.1 g test material. The solution was shaken for 10 minutes and examined for any undissolved material.

For the definitive study, aqueous samples were extracted into toluene and analyzed by gas chromatography using a flame ionization detector. Samples were quantified against standards of test substance in toluene, prepared from an acetone stock (Appendix 1, Appendix Figure 1). In the final definitive study, approximately 2 ml samples were centrifuged at 14000 rpm for 10 minutes at 20C. A 0.5 ml aliquot was removed by pipette and diluted with deionized water in a 100 ml volumetric flask. A 5 ml aliquot was removed by pipette and placed in a stoppered tube containing 5 ml toluene. The tube was shaken for 2 minutes, and a toluene aliquot was analyzed by gas chromatography. The methods were the same as mentioned above.

Water solubility was measured at 3 nominal concentrations of 5,000, 10,000 and 100,000 mg/L. For the lowest concentration, the solution was prepared at the beginning of the study to be held at 30C then equilibrated at 20C for 24 hours.

For the nominal concentration of 10,000 mg/L, the solution was maintained at 20C. This was analyzed at 0 hours and regular intervals thereafter.

In the final definitive run at 100,000 mg/L, the solution was maintained at 20C. Samples were analyzed at 0, 2.5, 5.5, 24.5, 30, 48, 53, 78, 144, 168, 191, 215.5, 239.5, 312.5, 340.5, 360, 383.5 and 407.5 hours.

Result : In the preliminary test, the apparent solubility appeared to be between 100 and 1000 mg/L. Therefore, the test solution concentration chosen for the definitive study was 5000 mg/L.

At the lowest nominal concentration, 5,000 mg/L, the measured concentrations showed a continual decrease due to hydrolysis (Table 1). For a nominal concentration of 5000 mg/L, measured values were 3631, 2321, 3092, 875, 1362, 1310, 1523, 608 and 204 mg/L for 24, 44, 52, 69, 76, 93, 101, 116 and 143 hours, respectively.

At 10,000 mg/L, the measured concentration showed that the test substance had gone fully into solution after 30 hours indicating that solubility must lie above 10,000 mg/L (Table 2, Figure 2).

In the final definitive run, the overall mean solubility on day 18 was 13,850 mg/L based on 19 measured values (Table 3, Figure 3). Individual values ranged from 10,600 to 17,360 mg/L.

The temperature of the 20C water bath ranged from 20.2 to 20.4C. During the definitive study, the pH measurements were all 7 for the nominal 100,000 mg/L test solution.

Attached document :

TABLE 1

MEASURED CONCENTRATIONS - RUN 1

Time (approx) hours	Measured concentration of nominal 5000 mg l^{-1} test vessels mg l^{-1}
24	3631
44	2321
52	3092
69	875.2
76	1362
93	1310
101	1523
116	608.4
143	204.0

Measured concentrations quoted to 4 significant figures

Each flask equilibrated for approximately 24 hours at 20°C, except for the 101 and 143 hour samples, equilibrated for 43 and 46 hours respectively, at 20°C

TABLE 2

MEASURED CONCENTRATIONS - RUN 2

Time	Measured concentration of nominal 10000 mg l^{-1} test vessel mg l^{-1}	
hours	Centrifuge at 3000 rpm	Centrifuge at 10000 rpm
0	689.5	738.0
2	5706	7487
4	9377	10230
6	11440	11180
22.5	10360	10510
25	10410	10910
28	10370	10270
30	10200	10120

Measured concentrations quoted to 4 significant figures

TABLE 3

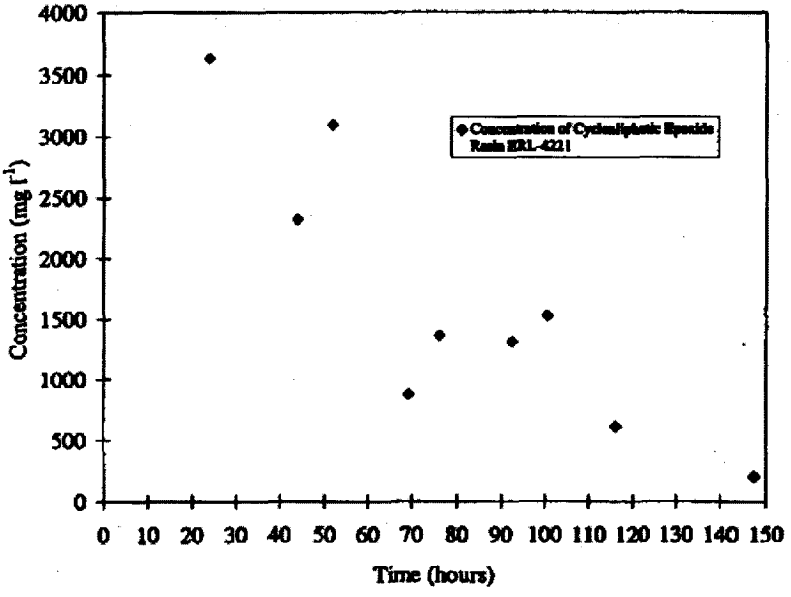
MEASURED CONCENTRATIONS - DEFINITIVE TEST RUN 3

Time hours	Measured concentration of nominal 100000 mg l^{-1} test vessel mg l^{-1}
0	12620
2.5	14090
5.5	14140
24.5	15170
30	14490
48	12470
53	12110
72	12720
78	17360
144	12910
168	13100
191	12820
215.5	14810
239.5	12770
312.5	10600
340.5	13880
360	15200
383.5	15970
407.5	15860

Measured concentrations quoted to 4 significant figures

FIGURE 1

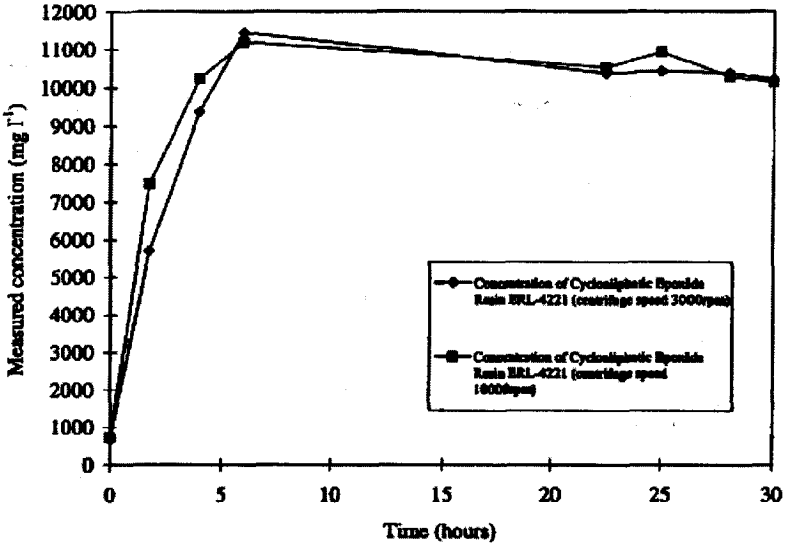
MEASURED CONCENTRATION VS TIME - RUN 1



Note Initial temperature 30°C, vessels equilibrated at 20°C for 24 hours (except for the 101 hour sample equilibrated for 43 hours)

FIGURE 2

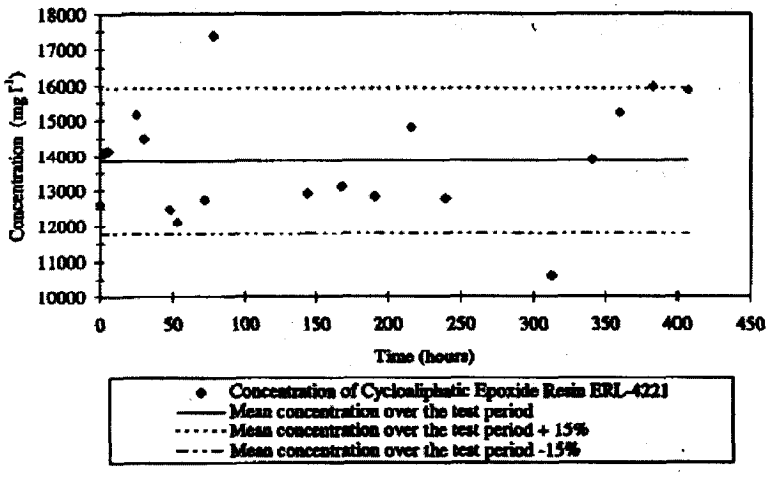
MEASURED CONCENTRATION VS TIME - RUN 2



Note Single vessel equilibrated at 20°C

FIGURE 3

MEASURED CONCENTRATION VS TIME
DEFINITIVE TEST RUN 3



Note Single vessel equilibrated at 20°C

Remark	:	The test material has been shown to hydrolyze in water. However, the rate of hydrolysis is slow enough, given the amount of excess material used in this study that it did not appear to interfere with the results.
Reliability	:	(1) valid without restriction 1a: GLP guideline study
23.07.2003		(6)
Solubility in	:	Water
Value	:	= 9798 mg/l at 15 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	OECD Guide-line 105
Year	:	1981
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	The solubility of ERL-4221 in glass distilled water and adjusted laboratory dilution water was determined. Test mixtures were prepared with an excess of test material and continuously mixed at 15C. Concentrations of the dissolved test substance were determined after mixing periods of 2, 5, 24 and 48 hours. Following mixing, test mixtures were centrifuged at 10,000 x g for up to 30 minutes to separate the solution into undissolved test material and a clarified supernatant fractions. The supernatant solutions were diluted with acetonitrile and analyzed in triplicate by liquid chromatography/mass spectrometry (LC/MS) using positive electrospray ionization (+ESI).
Result	:	The concentration of the test material in the clarified aqueous phase of the test mixtures averaged 9798 +/- 484 mg/L in glass distilled water and 12,671 +/- 690 mg/L in ALDW. Due to the known hydrolysis of this material in water, the test mixtures

were not mixed at 30C as recommended in the test guideline. Rather, the mixtures were tightly sealed and placed on a shaker bed within a 15C incubator for up to 48 hours.

At the conclusion of the experiment the pH of the aqueous test mixtures averaged 5.0 for the glass distilled water mixtures and 6.3 for ALDW mixtures.

A white preicipitate formed in the clarified ALDW test mixture at 48 hours, which was suspected to be the result of supersaturation in the solutions by the test substance. Additional centrifugation of these ALDW mixtures was performed to attempt to pellet the test material. No change in the measured solubility of the test material was observed. While the identity of the white material could not be confirmed, it is not expected to be due to hydrolysis products. Hydroxylated analogs would be expected to exhibit higher water solubility than that of the parent material. Prior characterization of ERL-4221 had shown that the test material contained several oligomers ranging in molecular weight from approximately 500 to 1300 amu. These oligomers comprised approximately 8% of the mixture. It is more likely that the white material is composed of these ether-linked oligomers already present in the test material.

Remark : The method used by Wallace, S.J. (2000). Cycloaliphatic epoxide resin ERL-4221: Determination of water solubility. Unpublished Union Carbide report 63-17 employed gas chromatography. Accelerated hydrolysis of the test substance may have occurred when the aqueous test solutions were injected into the heated injection port/column. In this study, LC-MS was used which minimized the test solution temperatures and thereby reduced the potential acceleration of hydrolysis.

Reliability : 1
1a: GLP guideline study

10.11.2005 (7)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Deg. product	:	
Method	:	
Year	:	2003
GLP	:	
Test substance	:	other TS: estimate conducted for pure double epoxy compound
Method	:	Used AOP v1.90 EPIWIN to calculate indirect photolysis.
Result	:	Estimated half-life for reaction with photochemically generated hydroxyl radical is 7.9 hours or 0.657 days.
Reliability	:	(2) valid with restrictions 2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment

08.12.2005

Type	:	water
Light source	:	Sun light
Light spectrum	:	ca. 290 - 800 nm
Relative intensity	:	based on intensity of sunlight
Conc. of substance	:	40 mg/l at °C
Deg. product	:	
Method	:	EPA OPPTS 835.2210
Year	:	2000
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	ERL-4221 has no absorbance in the range of 290-800 nm indicating that no direct photolysis takes place in sunlight.
Reliability	:	(2) valid with restrictions 2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment

08.12.2005 (8)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	= 21 hour(s) at 20 °C
t1/2 pH7	:	= 47 hour(s) at 20 °C
t1/2 pH9	:	= 42 hour(s) at 20 °C
Deg. product	:	
Method	:	other: follow parts of OECD 111
Year	:	1981
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Nominal solutions of 5000 mg/L were prepared in deionized water and remained at room temperature, 20C. Samples were analyzed at 0 and 72 hours. Aqueous samples were extracted into toluene and analyzed by gas chromatography using a flame ionization detector. Samples were quantified against standards of test substance in toluene, prepared from an acetone stock (Appendix 1, Appendix Figure 1). In addition, as part of the solubility determination, nominal concentrations of 5000 mg/L were prepared in deionized water at a pH slightly above 7,

held at 30C for 24 hours and then at 20C for up to 143 hours. The concentration of test material was determined at various time points between 24 and 143 hours.

Result : The analytical concentration of test material was determined at 0 and 72 hours at pH 4, 7 and 9 (Table 1). Based on this data, the half life at pH 4, 7 and 9 is 21, 47 and 42 days, respectively.

Table 1

pH	Analytical Conc. 0 hour	72 hours	Percentage loss (%)
4	33	3.0*	91
7	49	17	65
9	59	18	69

Nominal concentrations of 50 mg/L were prepared
* limit of detection.

The above data is confirmed with data obtained from the water solubility experiment (Table 2). At an approximate pH of 7.3, the half life was approximately 2 days.

Table 2

Time	pH	Analytical Conc. mg/L	Percentage loss (%)
24	7.14	3630	27
44	7.48	2320	54
52	7.36	3090	38
69	7.30	875	82
76	7.36	1360	73
93	7.32	1310	74
101	7.25	1520	70
116	7.32	610	88
143	7.26	200	96

Attached document : FIGURE 1

LOSS IN CONCENTRATION OF CYCLOALIPHATIC EPOXIDE RESIN ERL-4221 AT pH 4, 7 AND 9, ANALYTICAL METHOD DEVELOPMENT

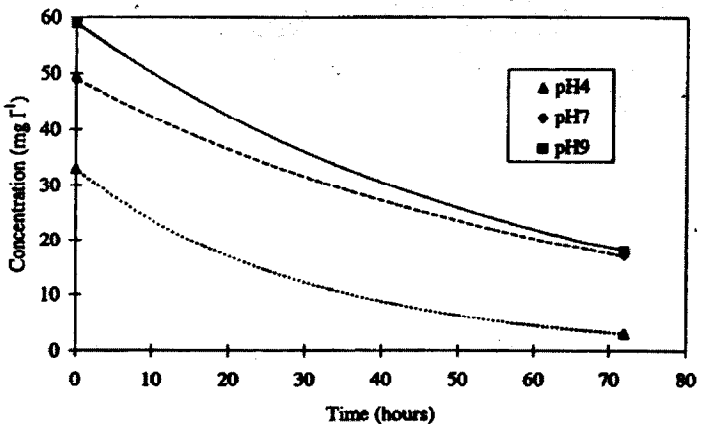


FIGURE 2

LOG CONCENTRATION VS TIME
ANALYTICAL METHOD DEVELOPMENT

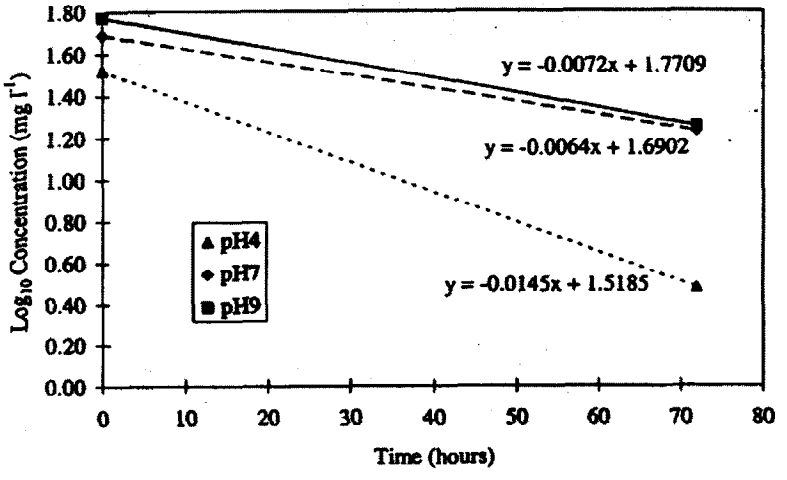


FIGURE 3

LOSS IN CONCENTRATION OF CYCLOALIPHATIC EPOXIDE RESIN ERL-4221
AT pH 7 RUN 1 OF SOLUBILITY STUDY

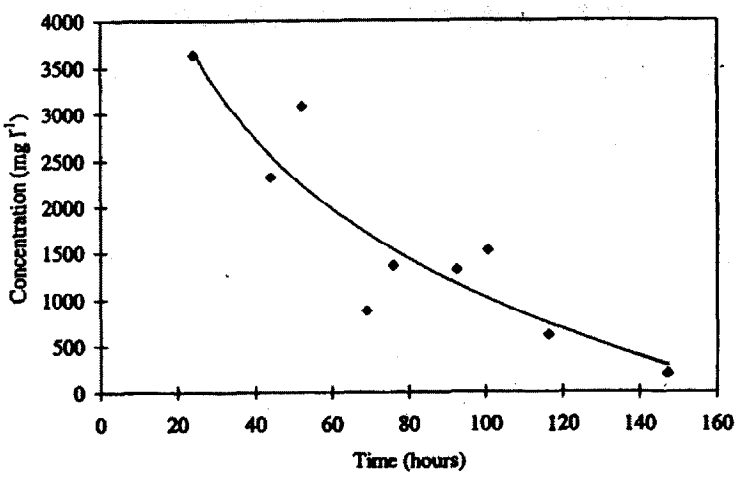
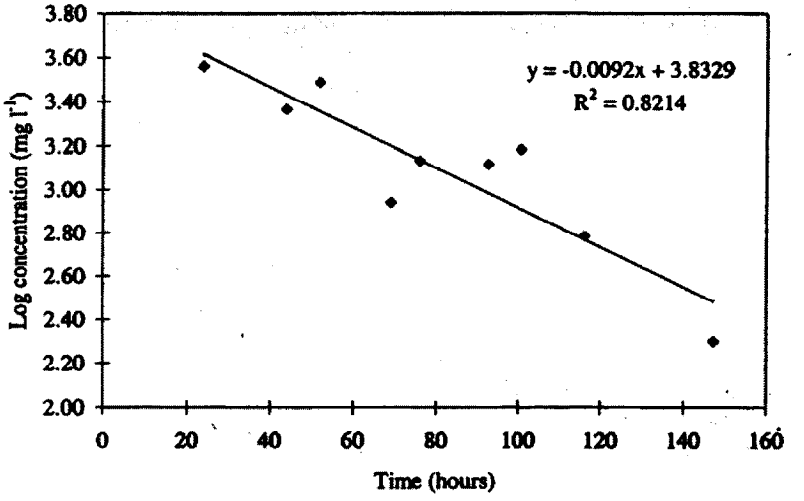


FIGURE 4

LOG CONCENTRATION VS TIME
RUN 1 OF SOLUBILITY STUDY



Remark : For this study, the report does not describe the analytical methods used. However, since the water solubility and octanol to water partition coefficient studies were conducted by the same author at the same time as this study, it seems reasonable to conclude similar analytical methods were used.

Reliability : (2) valid with restrictions
2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment

08.12.2005 (9)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : fugacity model level I
Media :
Air : .0007 % (Fugacity Model Level I)
Water : 98.1 % (Fugacity Model Level I)
Soil : 1.9 % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method :
Year : 2003

Method : Level I model version 2.11. Obtained from the Canadian Environmental Modeling Center, Trent University, Peterborough, Ontario, Canada

Property	Value	Source
Data Temperature (°C)	25	
Chemical Type	1	Type 1 indicates chemical can partition into all environmental compartments
Molecular Mass (g/mol)	252.31	Calculated from molecular structure
Water Solubility (g/m^3)	13,850	Measured value [1]
Vapor Pressure @ 25 ° C (Pa)	0.002	Measured value [1]
Melting Point (°C)	-32.5	Measured value [1]
Estimated Henry's Law Constant (H) ($Pa\ m^3/mol$)	3.6×10^{-5}	Calculated by Level I Fugacity Model [2]
Log K_{ow} Octanol-Water Partition Coefficient	1.34	Measured value [1]
Amount of Chemical input to Level I Model (kg)	100,000	Level I Default Value [2]

REFERENCES

1. Data from The Dow Chemical Company present in dossier.
2. Mackay, D., 2001. Multimedia Environmental Models: The Fugacity Approach. Lewis Publishers, CRC Press, Boca Raton, FL. Models available at: http://www.trentu.ca/cemc/models.html

Result : Media: Distribution among air, water, soil, and sediments

Emission Scenario	Percentage and amount distributed to				Residence Time (days) [without advection in brack
	Air	Water	Soil	Sediment	
Level I:	7.2×10^{-4} %	98.1 %	1.9 %	4.2×10^{-2} %	N/A
100,000 kg total emissions	7.2×10^{-1} kg	9.8×10^{4} kg	1.9×10^{3} kg	4.2×10^{1} kg	

Reliability : (2) valid with restrictions
2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment

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Type : fugacity model level III
Media :
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method :
Year : 2003

Method : Remarks: Level III model version 2.70. Obtained from the Canadian Environmental Modeling Centre, Trent University, Peterborough, Ontario, Canada

Property	Value	Source
Data Temperature (°C)	25	
Chemical Type	1	Type 1 indicates chemical can partition into all environmental compartments
Molecular Mass (g/mol)	252.31	Calculated from molecular structure
Water Solubility (g/m^3)	13,850	Measured value [1]
Vapor Pressure @ 25 ° C (Pa)	0.002	Measured value [1]
Melting Point (°C)	-32.5	Measured value [1]
Estimated Henry's Law Constant (H) ($Pa\ m^3/mol$)	3.6×10^{-5}	Calculated by Level I Fugacity Model [2]
Log K_{ow} Octanol-Water Partition Coefficient	1.34	Measured value [1]
Amount of Chemical input to Level I Model (kg)	100,000	Level I Default Value [2]
Reaction Half-lives (hr.) Input to Level III Model		
Air (vapor phase)	7.9	Estimated value [3]
Water (no susp. solids)	1,200	Based on ready biodegradability test result[1]*
Soil	2,160	Based on ready biodegradability test result [1]*
Sediment	2,160	Based on ready biodegradability test result[1]*
Suspended Sediment	$*1.0 \times 10^{11}$	Not expected to adsorb to susp. sediment
Fish	$*1.0 \times 10^{11}$	No uptake/bioaccumulation is expected
Aerosol	$*1.0 \times 10^{11}$	Aerosol emissions not expected

*Biodegradation exceeded 60% in 28 days, but not within the 10-day window; half-lives extrapolated from ready biodegradability test result according to Technical Guidance Document of the European Commission [4].
**Default value used in Level III model when reaction is expected to be negligible in this compartment

REFERENCES
1. Data from The Dow Chemical Company present in dossier.
2. Mackay, D., 2001. Multimedia Environmental Models: The Fugacity Approach. Lewis Publishers, CRC Press, Boca Raton, FL. Models available at: http://www.trentu.ca/cemc/models.html
3. U.S. EPA. 2000. AOPWIN software, version v1.90. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, D. C. Available at: http://www.epa.gov/oppt/exposure/docs/episuitedl.htm
4. European Commission. 1996. Technical Guidance Documents in support of the commission directive 93/67/EEC on risk assessment for new notified substances and commission regulation. European Commission, Brussels, Belgium.

Result : Media: Distribution among air, water, soil, and sediments

Emission Scenario	Percentage and amount distributed to				Residence Time (days) [without advection in brackets]
	Air	Water	Soil	Sediment	
Level III: 1,000 kg/hr to Air	2.6×10^{-3} % 3.1×10^{1} kg	39.4 % 4.7×10^{5} kg	60.5 % 7.3×10^{5} kg	1.9×10^{-2} % 2.3×10^{2} kg	1,203 [2,358]
Level III: 1,000 kg/hr to Water	1.1×10^{-6} % 7.1×10^{-5} kg	100 % 6.3×10^{5} kg	2.7×10^{-4} % 1.7 kg	4.8×10^{-4} % 3.0×10^{2} kg	634 [1,732]
Level III: 1,000 kg/hr to Soil	2.7×10^{-6} % 3.4×10^{-2} kg	38.1 % 4.8×10^{5} kg	61.9 % 7.7×10^{5} kg	1.8×10^{-2} % 2.3×10^{2} kg	1,251 [2,389]
Level III: 1,000 kg/hr simultaneously to Air, Water, and Soil	**9.9×10^{-4} % 3.1×10^{1} kg**	**51.3 % 1.6×10^{6} kg**	**48.7 % 1.5×10^{6} kg**	**2.5×10^{-2} % 7.6×10^{2} kg**	**1,029 [2,206]**

Conclusion : This material has high water solubility, low vapor pressure, and low log Kow. These properties dictate that the material has low potential to volatilize from water or soil to air, or adsorb from water to soil and sediments. When released to water, the material will remain dissolved in water and be removed through hydrolysis and biodegradation. When released to soil, the material will remain dissolved in soil pore water, and be removed through hydrolysis and biodegradation.

Reliability : (2) valid with restrictions
2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment

08.12.2005

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type : aerobic
Inoculum : activated sludge, domestic
Contact time :
Degradation : = 71 (±) % after 28 day(s)
Result :
Deg. product :
Method : OECD Guide-line 301 B "Ready Biodegradability: Modified Sturm Test (CO_2 evolution)"
Year : 1999
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : The inoculum used was activated sludge obtained from Newton Abbot sewage treatment works in England. The sewage treatment plant treats sewage predominantly of domestic origin. This was washed by centrifugation and re-suspension in test medium and maintained, aerated

at room temperature until required.

Result : After 3, 6, 10, 20 and 28 days, 0, 8, 28, 56 and 71% of the available ERL-4221 was biodegraded, respectively. Although 71% of ERL-4221 was biodegraded within 28 days it did not achieve the pass window of 60% in 10 days. Thus, although ERL-4221 undergoes biodegradation, it does not meet the formal definition of readily biodegradable.

Reliability : (1) valid without restriction
1a: GLP guideline study

08.12.2005 (10)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	flow through
Species	:	Oncorhynchus mykiss (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	= 3.2 measured/nominal
LC100	:	= 32 measured/nominal
Limit test	:	
Analytical monitoring	:	yes
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	2000
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : A stock concentrate was prepared at 320 g/L of ERL-4221 in dimethylformamide (DMF) and diluted by the appropriate amount of DMF to give a 300 ml solution at the correct nominal concentrations. The resultant test solutions were clear and colorless up to 100 g/L, clear and very pale yellow in color at 180 g/L and clear and pale yellow in color at 320 g/L.

Stock solutions were prepared from the stock concentrate. Stock solutions were further diluted to produce the desired concentrations fish were exposed to. Nominal concentrations fish were exposed to was 0 (control), 1.8, 3.2, 5.6, 10, 18 and 32 mg/L.

Result : For nominal concentrations of 1.8, 3.2, 5.6, 10, 18 and 32 mg/L the mean measured concentration at 0, 48 and 96 hours was 2.7, 2.9, 5.4, 10, 19 and 31 mg/L, respectively. Except for the lowest concentration, all values were within 10% of the target concentrations. The reason for the high analytical result for the lowest concentration of ERL-4221 is unknown.

At the highest analytical concentration, 31 mg/L, all animals died within 96 hours. At all lower concentrations, all animals survived. No clinically visible effects were noted at analytical concentrations of 2.7 and 2.9 mg/L. At 5.6 mg/L, dark discoloration was noted in the fish. At 10 mg/L, dark discoloration and sounding was noted. At 18 mg/L, dark discoloration, sounding, irregular respiration, surfacing and quiescense were reported.

Thus, the 96 hour LC50 was calculated to be 24 mg/L and the NOEC was 3.2 mg/L based on nominal concentrations.

The pH values ranged from 7.4 to 7.7, dissolved oxygen concentrations ranged from 9.8 to 10.4 mg/L, and the temperatures recorded were within the range 15+/-1C. The total hardness (as CaCO3) 45.7-48.3 mg/L and conductivity at 25C was 208-214 uS/cm.

Remark : ERL-4221 is rapidly hydrolyzed in water.

Reliability : (1) valid without restriction
1a: GLP guideline study

08.12.2005 (11)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
Analytical monitoring	:	yes

Method	:	OECD Guide-line 202
Year	:	1984
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Groups of 5 Daphnia magna/replicate were tested in 4 replicates to nominal concentrations of 0 (control), 5.6, 10, 18, 32, 56, 100 and 180 mg/L. Fresh test solutions were prepared at 0 and 24 hours. The test solution temperature was maintained at 20+/-1C. A photoperiod of 16 hours light:8 hours dark with 20 minute transition periods was provided. The test solutions were not aerated. Daphnia were not fed during the course of the study. Due to differences between nominal and analytical concentrations at the two highest concentrations, data from these two concentrations were excluded when calculating the 48-hour EC50 using Stephan's method. Stephan, C.E. (1977). Methods for calculating an LC50. In Aquatic Toxicology and Hazard Evaluation. Mayer, F.L. and Hamelink, J.L editors. Proceedings 1st Annual Symposium on Aquatic Toxicology. ASTM, 1977, STP 634 65-8.
Result	:	The nominal concentrations of 5.6, 10, 18, 32, 56, 100 and 180 mg/L resulted in mean analytical concentrations of 5.2, 9.4, 16, 28, 57, 101 and 160 mg/L, respectively, based on arithmetic means of the 0, 24 and 48 hour concentrations. The resultant solutions were clear and colorless except for the nominal 100 mg/L concentration where a slight film was visible on the surface and 180 mg/L with a slight film on the surface and fine white globules on the base of the test vessel. After 24 hours, 2 daphnia at 180 mg/L were immobilized. After 48 hours, the number immobilized was 0, 0, 0, 2, 10, 11, 1 and 4 for nominal concentrations of control, 5.6, 10, 18, 32, 56, 100 and 180 mg/L, respectively. The 48 hour NOEC was 10 mg/L and the 48-hour EC50 was 40 mg/L based on nominal concentrations. The EC100 was not determined. Dissolved oxygen concentrations ranged from 8.8 to 9.2 mg/L and the pH values ranged from 7.84 to 8.05. The mean total hardness of the reconstituted dilution water batches was 609 mg/L CaCO3. Note Appendix 3 lists the hardness as 221 mg/L CaCO3 and the conductivity as 609 uS/cm at 25C.

Attached document :

TABLE 1

ANALYTICAL RESULTS[a]

Nominal conc of Cycloaliphatic Epoxide Resin ERL-4221	Measured conc of Cycloaliphatic Epoxide Resin ERL-4221 ($mg\ l^{-1}$)										Mean measured conc over the test duration[b]	Mean measured conc as % of nominal	% Loss in conc over the test duration[c]
	0 hour		24 hours 'off'		24 hours 'on'		48 hours 'off'						
($mg\ l^{-1}$)	uncent	cent	uncent	cent	uncent	cent	uncent	cent	(uncent)	($mg\ l^{-1}$)	(uncent)	(uncent)
Dilution water control	< 0.27	< 0.27	< 0.090	< 0.090	< 0.090	< 0.090	< 0.037	< 0.037	< 0.27	-	-	-
5.6	5.3	5.3	5.7	4.8	5.1	5.5	4.5	4.4	5.2	93	<1 - 12	
10	9.7[d]	9.5[g]	9.8[e]	9.6[h]	9.9[f]	10[i]	8.2	8.6	9.4	94	<1 - 17	
18	17	17	15	15	18	17	15	15	16	89	12 - 17	
32	30	30	28	29	28	27	27	28	28	88	4 - 7	
56	57	57	51	49	64	60	57	57	57	102	11	
100	110	120	97	92	100	100	95	92	101	101	5 - 12	
180	200	210	110	98	180	180	150	150	160	89	17 - 45	

a All measurements are quoted to 2 significant figures and percentages are quoted to the nearest integer
b Calculated using the arithmetic mean of the 0, 24 and 48 hour results
c 100 - ((final analysed concentration 'off'/concentration 'on') × 100), quoted as a range for the two sets of solutions
d Mean of triplicate analyses: 9.9, 9.7 and 9.5 $mg\ l^{-1}$
e Mean of triplicate analyses: 9.4, 10 and 10 $mg\ l^{-1}$
f Mean of triplicate analyses: 10, 9.9 and 9.8 $mg\ l^{-1}$
g Mean of triplicate analyses: 9.7, 9.3 and 9.4 $mg\ l^{-1}$
h Mean of triplicate analyses: 11, 9.0 and 8.7 $mg\ l^{-1}$
i Mean of triplicate analyses: 10, 10 and 10 $mg\ l^{-1}$
NB Uncent and cent are uncentrifuged and centrifuged samples respectively.

Fortification samples:

0 hours,	8:1 extraction: Mean recovery	103% (99, 99, 110%)
0 hours,	1:1 extraction: Mean recovery	95% (93, 93, 99%)
24 hours,	8:1 extraction: Mean recovery	103% (100, 110, 100%)
24 hours,	1:1 extraction: Mean recovery	97% (95, 93, 100%)
48 hours,	8:1 extraction: Mean recovery	99% (99, 98, 99%)
48 hours,	1:1 extraction: Mean recovery	97% (103, 95, 90%)

TABLE 2

Daphnia RESPONSE

Time (hours)	Nominal conc of Cycloaliphatic Epoxide Resin ERL-4221 ($mg\ l^{-1}$)	Number immobilised per replicate				Total number tested	Total number immobilised	% Immobilised
		A	B	C	D			
24	Dilution water control	0	0	0	0	20	0	0
	5.6	0	0	0	0	20	0	0
	10	0	0	0	0	20	0	0
	18	0	0	0	0	20	0	0
	32	0	0	0	0	20	0	0
	56	0	0	0	0	20	0	0
	100	0	0	0	0	20	0	0
	180	1	0	0	1	20	2	10
48	Dilution water control	0	0	0	0	20	0	0
	5.6	0	0	0	0	20	0	0
	10	0	0	0	0	20	0	0
	18	0	0	0	2	20	2	10
	32	2	4	2	2	20	10	50
	56	2	3	3	3	20	11	55
	100	1	0	0	0	20	1	5
	180	2	0	0	2	20	4	20

APPENDIX 2

ANALYTICAL METHOD FOR THE DETERMINATION OF CYCLOALIPHATIC EPOXIDE RESIN ERL-4221 IN WATER SAMPLES

Aqueous samples of Cycloaliphatic Epoxide Resin ERL-4221 were extracted into toluene and analysed by gas chromatography using a flame ionisation detector. The samples were quantified against standards of test substance in toluene, prepared from an acetone stock.

GC Conditions

Column	25 mm × 0.32 mm (id) 25 μm film thickness
Column packing	CP-Sil-8CB
Column temperature	

	Start temp °C	Ramp rate °C min^{-1}	Final temp °C	Hold time min
initial	60	-	-	2.0
prgm 1	60	20	220	7.0

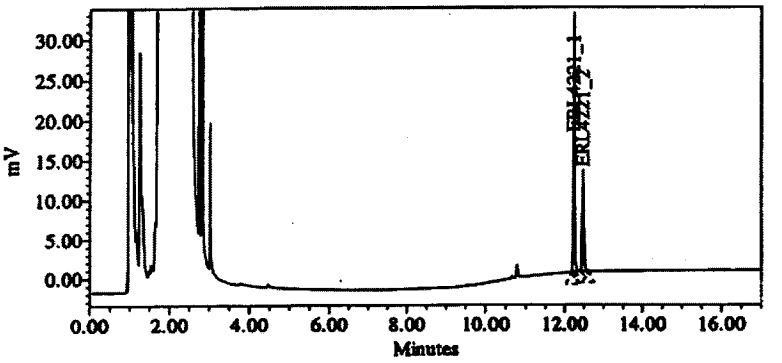
Injection port temperature	250°C
Injection volume	2 μl
Splitter ratio	100:1 open after 0.75 min
Carrier gas flow rate	helium @ 2.0 ml min^{-1}
Detector	flame ionisation
Detector gases	nitrogen make up @ 35 ml min^{-1}
	air @ 300 ml min^{-1}
	hydrogen @ 25 ml min^{-1}
Detector temperature	300°C
Detector range	12

Using the above conditions, two peaks were obtained for Cycloaliphatic Epoxide Resin ERL-4221 with retention times of approximately 12.3 and 12.5 minutes. A 100 mg l^{-1} standard of Cycloaliphatic Epoxide Resin ERL-4221 in toluene produced approximately a total peak area (sum of two peaks) of 1.2 × 10^{5} μV s using a Millennium^{32} (version 3.05.01) chromatographic data system.

APPENDIX 2 FIGURE 1

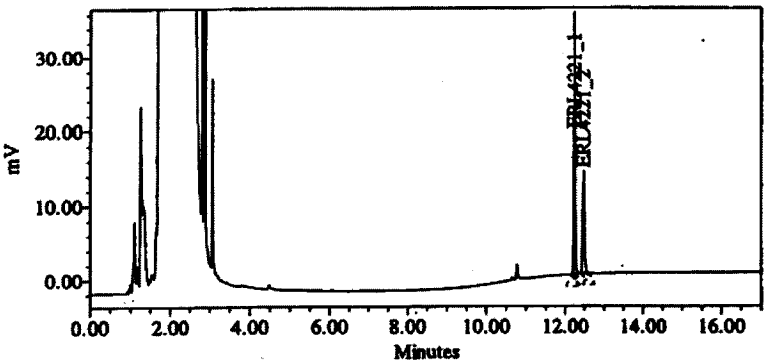
TYPICAL CHROMATOGRAMS

(A) 100 mg l^{-1} standard concentration of Cycloaliphatic Epoxide Resin ERL-4221



Unique_Number Dec14_79 Injection 1 SampleName S100 Date Acquired 17/12/99 15:18:40

(B) 100 mg l^{-1} nominal concentration of Cycloaliphatic Epoxide Resin ERL-4221



Unique_Number Dec14_78 Injection 2 SampleName (41) 100 mg/l - Cent.(K) Date Acquired 17/12/99 14:53:54

Remark : Although Wallace, S.J. (2000) Cycloaliphatic epoxide resin ERL-4221: Hydrolysis as a function of pH by estimation. Unpublished UCC report 63-74 reported a half life of 47 hours, subsequent analysis by other analytical methods demonstrated only 20% parent was lost due to hydrolysis in a 48 hour period. This is thought to be due to gas chromatographic analysis conducted at high temperatures by Wallace (2000).

Whether one uses the nominal or analytical concentration values, the 48 hr EC50 value is greater than 18 or 16 mg/L, respectively. The NOEC is 10 mg/L based on nominal concentration.

Although the authors state the test was conducted under static conditions, the authors also state the test material was prepared at 0 and 24 hours. Thus this was a static renewal test.

Reliability : (1) valid without restriction
1a: GLP guideline study

08.12.2005 (12)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Selenastrum capricornutum (Algae)
Endpoint	:	biomass
Exposure period	:	72 hour(s)
Unit	:	mg/l
NOEC	:	= 22 measured/nominal
LOEC	:	= 27 measured/nominal
EC50	:	= 90 measured/nominal
Limit test	:	
Analytical monitoring	:	yes
Method	:	OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year	:	2000
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : Nominal exposure concentrations of 0 (control), 5.6, 10, 18, 32, 56, 100 and 180 mg/L were used.

Result : For the nominal concentrations of 5.6, 10, 18, 32, 56, 100 and 180 mg/L, the geometric mean measured concentration was 3.7, 6.6, 11, 22, 30, 27 and 110 mg/L, respectively, based on values obtained at 0 and 72 hours. The percent loss of ERL-4221 during the 72 hours was 46, 49, 53, 47, 48, 94 and 72% for nominal concentrations of 5.6, 10, 18, 32, 56, 100 and 180 mg/L, respectively.

For the measured concentrations of 0(control), 3.7, 6.6, 11, 22, 30, 27 and 110 mg/L, the average algal cell density from six replicates at 72 hours was 99.3, 99.1, 90.6, 97.0, 118, 104, 67.7 and 19.6 cells/ml (x10+4), respectively. The no-observed-effect-concentration (NOEC) was 22 mg/L and the lowest significant effect concentration was 27 mg/L

At the start of the test the pH of the test solutions ranged from 7.3 to 7.4 and at the end of the test the range was 7.3 to 8.2. During the course of the test the pH of the control culture medium solutions increased by 0.4 units.

Daily temperature measurements ranged from 24.1 to 24.3C.

The light intensity, measured once during the study, was 8040 lux.

Remark : ERL-4221 is rapidly hydrolyzed in water.

The analytical data for the three highest concentrations is not consistent.

Table 1

Measured concentration of ERL-4221

Nominal	Analytical Conc, mg/L	
Conc	0 hr	72 hr
56	42	20
100	110	6.5
180	190	55

Data is based on centrifuged values.

The half life, based on hydrolysis is expected to be 1-2 days. Thus one would expect approximately 75% of the material to be hydrolyzed within 72 hours. The difference noted at 72 hr for the nominal concentration of 100 mg/L is probably due to an analytical error.

Reliability : (1) valid without restriction
1a: GLP guideline study

08.12.2005

(13)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	ca. 5000 mg/kg bw
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	20
Vehicle	:	
Doses	:	
Method	:	OECD Guide-line 401 "Acute Oral Toxicity"
Year	:	1999
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Groups of 5 male and 5 female rats were dosed via oral gavage with 2959 and 5000 mg/kg.
Result	:	Three males and two females in the 5000 mg/kg group died within six days of dosing. Mortality was 0/10 and 5/10 for the 2959 and 5000 mg/kg groups, respectively. Clinical findings were noted in both dose groups during the first week of the study. Four animals in the 2959 mg/kg group and all animals in the 5000 mg/kg group were noted with various discolored areas due to discharges/excretions, hypoactivity and/or impaired muscle coordination. Three animals in the 5000 mg/kg group were noted with decreased defecation, decreased urination, labored respiration and/or convulsions. There were no other clinical findings. All surviving animals appeared normal by day 6 and throughout the remainder of the study. There were no remarkable body weight changes observed during the study. Three animals that died were noted with gastric abnormalities. There were no other internal gross necropsy findings for animals found dead. At the terminal necropsy, findings included capsular scarring on the spleen for one male in the 5000 mg/kg group. There were no other findings for any examined tissues at the scheduled necropsy. The LD50 of cycloaliphatic epoxy resin ERL-4221 was found herein to be approximately 5000 mg/kg in fasted male and female Sprague Dawley rats when administered once orally via gavage.
Remark	:	The specific gravity of the test material was 1180 mg/ml. Thus 5000 mg/kg corresponds to 4.24 ml/kg which is in very close agreement with the study conducted in 1961.
Reliability	:	(1) valid without restriction 1a: GLP guideline study

08.12.2005 (14)

Type	:	LD50
Value	:	= 4.49 ml/kg bw
Species	:	rat
Strain	:	
Sex	:	male

Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	
Year	:	1961
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Groups of 5 male rats were dosed orally with 2.0, 4.0, 8.0 or 16.0 ml/kg and observed for 14 days. Gross pathologic exam was performed on animals that died. Animals were weighed prior to dosing and survivors were weighed 14 days later. The method of moving average for calculating the median-effective dose was applied to the 14-day mortality data.
Result	:	Animals weighed between 99 and 120 grams on the day of dosing. The number of animals dying was 2, 2, 3 and 5 at doses of 2.0, 4.0, 8.0 and 16.0 ml/kg. Deaths in the highest dose group occurred within 18 hours after dosing while most of those on the lower levels were delayed from two to four days. Gross pathological findings included congestion of the lungs, stomachs, intestines and adrenals; paleness of the kidneys; and congestion of the livers with priminent acini and burned areas discernible on surfaces that had been in opposition to stomachs that still contained part of the dose. Animals that survived gained between 58 and 110 grams. The oral LD50 was 4.49 ml/kg.
Remark	:	The specific gravity of the test material was 1180 mg/ml. The oral LD50 of 4.49 ml/kg corresponds to 5300 mg/kg which is in very close agreement with the study conducted in 1999.
Reliability	:	(2) valid with restrictions 2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment

08.12.2005 (15)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LC50
Value	:	
Species	:	rat
Strain	:	
Sex	:	female
Number of animals	:	6
Vehicle	:	
Doses	:	
Exposure time	:	8 hour(s)
Method	:	
Year	:	1961
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	A group of 6 female rats was exposed to saturated vapors of EP-221 for 8 hours. Saturated vapors were generated at a temperature of 21C by passing dried air at the rate of 2.5 liters/minute through a fritted glass disc immersed to a depth of at least one inch in 50 ml of EP-221. The animals were observed for 14 days and a gross necropsy exam was conducted on surving animals as well as those that died during the observation period.
Result	:	All animals survived the 8 hour exposure period as well as the 14 day post exposure observation period. The animals gained weight during the subsequent two week observation period and exhibited no grossly visible effects upon gross examination.
Remark	:	Based on the low vapor pressure, 0.00002 hPA, a saturated atmosphere contains approximately 0.02 ppm EP-221.

Reliability	:	(2) valid with restrictions 2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment
08.12.2005		(15)
Type	:	LC50
Value	:	
Species	:	rat
Strain	:	
Sex	:	female
Number of animals	:	6
Vehicle	:	
Doses	:	
Exposure time	:	8 hour(s)
Method	:	
Year	:	1961
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	A group of 6 female rats was exposed to a condensation aerosol and any decomposition products of EP-221 for 8 hours. The condensation aerosol was generated by passing dried air at the rate of 2.5 liters/minute through a fritted glass disc immersed to a depth of at least one inch in 50 ml of EP-221. The glass disc was submerged in a silicone oil bath maintained at a temperature sufficiently high to keep the EP-221 at approximately 162C. The ambient air temperature in the chamber never exceeded 28C. The animals were observed for 14 days and a gross necropsy exam was conducted on surving animals as well as those that died during the observation period.
Result	:	Five of six rats survived after eight hours in this atmosphere. The lone death occurred the day after inhalation and autopsy revealed from 70 to 80% lung hemorrhage. The five survivors gained weight normally during the subsequent two week observation period. There were no grossly visible changes observed in any of the surviving rats at the conclusion of the two week observation period.
Reliability	:	(2) valid with restrictions 2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment
08.12.2005		(15)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Value	:	> 20 ml/kg bw
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male
Number of animals	:	4
Vehicle	:	
Doses	:	
Method	:	
Year	:	1961
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	A group of 4 male New Zealand White rabbits, 3-5 months of age and averaging 2.5 kg were dosed dermally with 20 ml/kg of ERL-4221. The hair on the trunk of the rabbits was removed prior to treatment. The test material was applied and a sheet of VINYLITE was placed on the application site to retain the dose in contact with the skin. The rabbits were

immobilized during the 24 hour dermal contact period. After 24 hours, the sheeting was removed and the animals were caged for the remainder of the 14-day observation period. Body weights were obtained prior to dosing and at the end of the observation period.

Result : One of four rabbits dosed dermally with 20 ml/kg died four days after dosing. The cause of death could not be determined. Two of three survivors lost weight (20 and 408 gram) during the two-week observation period.

No additional information supplied in report.

Reliability : (2) valid with restrictions
2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment

08.12.2005 (15)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Exposure	:	
Exposure time	:	4 hour(s)
Number of animals	:	6
Vehicle	:	
PDII	:	
Result	:	
Classification	:	
Method	:	OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"
Year	:	1992
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : A group of 6 rabbits, 3 males and 3 females, was used. A volume of 0.5 ml was applied to the dorsal area of the trunk of each rabbit which had been previously clipped free of hair. A 1-inch square gauze patch was placed over the dose site and was secured by adhesive tape. Polyethylene sheeting was placed loosely around the trunk and secured. The animal was restrained for the 4-hour contact period after which the coverings and excess test material were removed. Readings were made starting at 1, 24, 48 and 72 hours and at 7 and 14 days after the 4 hour contact period using the Draize method (Draize, 1959).

Draize, J.H. (1959). The appraisal of chemicals in foods, drugs and cosmetics. The Association of Food and Drug Officials of the United States.

Result : Application of 0.5 ml of Cyracure UVR-6110 to covered rabbit skin for a 4-hour contact period produced minor erythema on 6 of 6 rabbits. Minor transient edema was produced on 3 animals. There was no edema present on any animal by 2 days. Erythema subsided on 5 of 6 rabbits within 7 days and the last rabbit within 14 days.

The modified primary irritation score (average of 1 and 24 hour mean values) was 1.35. This material is not irritating or corrosive to skin.

Reliability : (1) valid without restriction
1a: GLP guideline study

08.12.2005 (16)

Species	:	rabbit
Concentration	:	
Exposure	:	Occlusive
Exposure time	:	24 hour(s)
Number of animals	:	6
Vehicle	:	
PDII	:	
Result	:	
Classification	:	
Method	:	
Year	:	1984
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Method : A group of 3 male and 3 female rabbits were dosed with 0.5 ml of test material applied to the clipped, intact skin under a gauze patch. The patch is loosely covered with impervious sheeting. The animals are restrained for the 24 hour contact period. Excess sample is removed after contact. Skin reaction is scored, by the method of Draize, at one day (shortly after removing the patch and impervious sheeting), 2 days, 3 days and, depending upon the local skin reaction, possibly 7, 10 and 14 days after dosing.

Result : There was no evidence of edema observed after the 24 hour application of test material. Very slight erythema was noted in 3 of 6 rabbits, 1 male and 2 females, shortly after removal of the patch containing test material. Approximately 24 hours after removing the patch very slight erythema was still noted in one male rabbit. Desquamation was noted in one and two male rabbits two and 6 days after removing the patch. The study was concluded on day 6 after removal of the patch.

CYRACURE Resin UVR-6110 produced minor irritation on rabbit skin following a 24-hour occluded application.

Reliability : (2) valid with restrictions
2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment

08.12.2005 (17)

Species	:	rabbit
Concentration	:	undiluted
Exposure	:	
Exposure time	:	
Number of animals	:	5
Vehicle	:	
PDII	:	
Result	:	
Classification	:	
Method	:	
Year	:	1961
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Method : Undiluted EP-221 was applied undiluted to clipped skin on the belly of the rabbit. The dosage was 0.01 ml.

No additional information provided.

Result : There was no reaction on four animals and marked capillary injection on a fifth.

No additional information provided

Remark : No information provided in the report on the length of the exposure to test material. The amount used, 0.01 ml, is much lower than currently required in OECD guidelines, 0.5 mls. Thus the study is considered to be invalid.

Reliability : (3) invalid
3b: Significant methodological deficiencies

08.12.2005 (15)

5.2.2 EYE IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Dose	:	.1 ml
Exposure time	:	
Comment	:	
Number of animals	:	4
Vehicle	:	
Result	:	
Classification	:	
Method	:	OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year	:	1992
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : A total of 4 rabbits, 2 males and 2 females, were used. A volume of 0.1 ml of test material was placed into the conjunctival sac of 1 eye/rabbit. The other eye of each animal served as the control. The eye was examined at 1, 24, 48 and 72 hours and at 7 and 9 days following instillation. Fluorescein staining was performed at day 1 and each subsequent examination. Grading and scoring followed the Draize system (Draize, 1959).

Draize, J.H. (1959). The appraisal of chemicals in foods, drugs and cosmetics. The Association of Food and Drug Officials of the United States.

Result : A volume of 0.1 ml of test material instilled into rabbit eyes produced no corneal injury or irits in any of 4 rabbits dosed. Minor conjunctival irritation consisting of slight erythema, slightly swollen conjunctiva and slight discharge, was observed in all 4 rabbits within 1 hour. The dosed eye of 1 rabbit healed within 48 hours. The 3 remaining rabbits had a normal ocular appearance within 72 hours to 9 days. The material is not an ocular irritant using the Draize/FHSA interpretation.

FHSA (1989). Consumer Product Safety Commission (1989). Regulations under FHSA, Title 16, Code of Federal Regulations, Ch. II, Commercial Practices, Sectoin 1500.

Reliability : (1) valid without restriction
1a: GLP guideline study

08.12.2005 (16)

Species	:	rabbit
Concentration	:	undiluted
Dose	:	.1 ml
Exposure time	:	
Comment	:	
Number of animals	:	6
Vehicle	:	
Result	:	
Classification	:	
Method	:	
Year	:	1984
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Method	:	A group of 3 male and 3 female rabbits were dosed with 0.1 ml of test material. The dose is instilled into the lower conjunctival sac of one eye per animal or is placed directly on the eye. The eyes are held together for one second. Six eyes are dosed per test volume. The eyes are scored at one and 4 hours and 1, 2 and 3 days after dosing. The eyes were scored using a modification of the Draize score.
Result	:	There was no observable effect on the cornea or iris at any time point post dosing. The most severe effects on the conjunctiva occurred one hour after dosing and all effects were absent within 48 hours. Conjunctival redness varied from 0 (normal) to 2 (diffuse, deep crimson red) one hour after dosing. The mean score was 1.5. By 24 hours after dosing, 2 rabbits still exhibited slight conjunctival redness. All animals appeared normal within 48 hours after dosing. The swelling ranged from 0 (normal) to 2 (obvious swelling with partial eversion of eyelids). The mean score was 0.8. By 24 hours there was no visible evidence of swelling. The eye discharge ranged from 0 (normal) to 2 (discharge moistening lids and hairs adjacent to lids). The mean score was 1.0. By 24 hours there was no visible evidence of a discharge. Instillation of 0.1 ml of sample into rabbit eyes produced minor transient irritation. All animals appeared normal within 48 hours after dosing.
Reliability	:	(2) valid with restrictions 2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment

08.12.2005 (17)

Species	:	rabbit
Concentration	:	undiluted
Dose	:	.5 ml
Exposure time	:	
Comment	:	
Number of animals	:	5
Vehicle	:	
Result	:	
Classification	:	
Method	:	
Year	:	1961
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Five rabbits had 0.5 ml of EP-221 instilled into the eye. No additional information provided.
Result	:	Three rabbit eyes were apparently unharmed and two others had only trace injuries.
Remark	:	Although the dose volume was much greater than currently recommended, the very slight irritation observed suggests minimal or no eye irritation at recommended dose volumes.
Reliability	:	(2) valid with restrictions 2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment

08.12.2005 (15)

5.3 SENSITIZATION

Type	:	Guinea pig maximization test
Species	:	guinea pig
Concentration	:	1st: Induction 5 % intracutaneous 2nd: Induction 100 % occlusive epicutaneous 3rd: Challenge 100 % occlusive epicutaneous

Number of animals	:	
Vehicle	:	other: propylene glycol was used for intracutaneous administration
Result	:	sensitizing
Classification	:	sensitizing
Method	:	other: essentially follows OECD 0406
Year	:	1991
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : Range finding study - Groups of 2 guinea pigs were administered intradermal injections (2 sites per animal) of a 5.0% v/v concentration of the test material in propylene glycol. Animals were observed at 24 and 48 hours for necrosis and alterations.

Range finding study - A topical range-finding study was performed to determine the lowest concentration which produced mild irritation (to be used for induction) and the highest concentration which did not produce irritation (to be used for challenge. Six guinea pigs, 3 male and 3 female were used. Each animal was dosed with 10, 25, 50% v/v and 100% test material. The vehicle used was 70% ethanol. A 0.1 ml sample of test material was placed on each application site and the sites were covered with plastic sheeting which was secured with elastic adhesive bandage. After 24 hours, the bandages, sheeting and patches were removed. Observations for signs of dermal irritation (erythema, edema and eschar formation) were made approximately 24 and 48 hours after removal of the patches.

Definitive study - A group of 10 male and 10 female guinea pigs were used in the guinea pig maximization test. For intradermal doses, a 5% concentration of ERL-4221 dissolved in propylene glycol was used. For the topical application neat ERL-4221 was used. Since the test material was non-irritating at 100% concentration, the area was pre-treated with 10% sodium lauryl sulfate (SLS) in petrolatum 24 hours before the material was applied in order to provoke a mild inflammatory reaction. The SLS was massaged into the skin with gloved fingers. An additional group of 5 male and 5 female guinea pigs were used as irritation controls. For the irritation controls, neat propylene glycol was used for intradermal doses while 70% ethanol was used topically.

For the challenge phase, 0.1 ml of test material was applied and remained on the skin for 24 hours on day 21 for animals from the definitive study and irritation controls. Dermal readings were made on all animals 24 and 48 hours after removal of the patches.

Result : Range finding study - For the intradermal injection, a 5.0% solution in propylene glycol was injected in two sites in two animals. Local necrosis was the only effect observed. There was no evidence of extensive necrosis or ulceration. Therefore this concentration was used for the intradermal induction administration.

Range finding study - For the topical application, six animals, 3 females and 3 males, were used. Concentrations of 10, 25 and 50% v/v and 100% were placed on one of four sites on each animal. The undiluted material was non-irritating and was therefore administered at a 100% concentration for both induction and challenge.

Definitive study - One male guinea pig died 4 days after the topical application. Gross postmortem observations revealed discolored lungs, the surface of liver was yellow and the abdominal cavity was filled with yellow fluid. Since all remaining animals were free of any signs of toxicity, this death was probably not related to test material administration. During the challenge phase 19 of 19 guinea pigs exhibited a score of 0.5 or greater and 12 of 19 had a score of 1.0. For the irritation control animals, 0 of 10

guinea pigs exhibited a score of 0.5 or greater.

Based on this study, ERL-4221 exhibited a potential to produce dermal sensitization in the guinea pig.

Reliability : (1) valid without restriction
1a: GLP guideline study

08.12.2005 (18)

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	other: CFE albino strain
Route of admin.	:	oral feed
Exposure period	:	97 days
Frequency of treatm.	:	daily
Post exposure period	:	
Doses	:	0, 31.25, 125, 500 and 2000 mg/kg/day
Control group	:	yes, concurrent no treatment
Method	:	
Year	:	1963
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Method : Groups of 10 male and 10 female rats were fed diets containing 0, 31.5, 125, 500 or 2000 mg/kg/day epoxide 221. Rats were fed twice weekly during the first week of the study and weekly thereafter. Animals were observed daily for symptoms of abnormalities. Animals that died were necropsied. Animals that survived to the end of the study were necropsied and liver and kidney weights were obtained.

Result : Mortality was not substantially affected at any dose level. None, one or two rats of each sex died during the three month period. All deaths, except one where autolysis concealed the cause, were attributed to lung infections.

Feed consumption was decreased at all dose levels for the females and at 0.125 and 2.0 mg/kg/day for the males. Similarly body weight gain for 125, 500 and 2000 mg/kg/day females was significantly lower than that of the controls, while only the 2000 mg/kg/day male group was affected.

Relative liver weights of the 2000 mg/kg/day male group and 500 mg/kg/day female group were significantly higher than control values. Kidney weights of 500 and 2000 mg/kg/day male and female groups were significantly increased. However, there were no gross or histopathological effects noted in the liver or kidney or any of the other organs examined from those of the control animals.

Reliability : (3) invalid
3b: Significant methodological deficiencies. Pulmonary infections affected survival in all groups.

08.12.2005 (19)

Type	:	Sub-acute
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	14 days
Frequency of treatm.	:	daily
Post exposure period	:	
Doses	:	0, 111, 556, 834 and 1113 mg/kg/day

Control group : yes, concurrent vehicle
LOAEL : = 100 mg/kg bw
Method :
Year : 2000
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : Groups of 10 male and 10 female rats received 0, 111, 556, 834 and 1113 mg/kg/day of ERL-4221 via oral gavage for 14 consecutive days. The vehicle for all dose levels was Mazola corn oil. In-life observations included clinical observations each day. Body weight and feed consumption were measured on a weekly basis. At the end of the study, the animals were fasted prior to necropsy. A complete necropsy was conducted on all animals. With two exceptions, a complete set of tissues were placed in 10% neutral buffered formalin. Testes were preserved in Bouin's solution and poles of the left kidney and one half of the right kidney and part of the left lobe of the liver were frozen and stored at -70C. The frozen kidney and liver were evaluated for 32P-postlabeling of DNA adducts (reported in in-vivo genetics section of dossier). Standard tissues were weighed at necropsy. Microscopic examination was conducted on the adrenal glands, kidneys, lungs, spleen, stomach and gross lesions from five randomly selected animals/sex/group in the control and 1000 mg/kg/day groups. The livers (males and females) and testes (males) were examined from each of the lower dose levels.

Statistical analysis of body weights, body weight changes, feed consumption and absolute and relative organ weights was conducted using a one-way analysis of variance (ANOVA) followed by Dunnett's test if the ANOVA revealed statistical significance ($p<0.05$).

Result : Test material-related clinical observations consisted of evidence of increased salivation in males and females receiving 500, 750 and 1000 mg/kg/day and yellow material in the urogenital area of females receiving 750 and 1000 mg/kg/day. These clinical signs were observed in some animals from each of the groups, most frequently, but not exclusively, at the 1-hour post-dosing interval. The incidence of wet yellow material observed in the urogenital area one-hour after dosing was 8 times in 4 high-dose females and the incidence of dried yellow material observed in the urogenital area one-hour after dosing was 4 times in 3 high-dose females. The total number of female rats in this group was ten and each rat was observed 9 times post-dosing for a total of 90 observations. Thus, although yellow material was observed on the fur, the frequency was quite low.There was no affect on survival. All rats survived until the scheduled sacrifice.

Dose-related decreases in body weight and weight gain were observed for males dosed at 500 mg/kg/day and above and for females at 1000 mg/kg/day (Table 1). Decreased mean feed consumption was observed in the 750 and 1000 mg/kg/day group males during the first week of the study.

Table 1

Body weights (g) of rats in range-finding study

	mg/kg/day				
Week	0	100	500	750	1000
	males				
0	291+/-24.4	291+/-24.7	287+/-22.1	286+/-27.1	287+/-21.6
1	323+/-30.7	323+/-34.1	306+/-29.7	296+/-37.9	299+/-24.7
2	354+/-37.3	353+/-38.8	333+/-31.4	321+/-41.4	316+/-27.1
	females				

0	197+/-16.7	198+/-12.9	198+/-15.0	202+/-16.6	200+/-14.0
1	214+/-14.5	212+/-17.8	210+/-14.3	215+/-17.6	208+/-16.4
2	229+/-15.2	225+/-19.2	226+/-19.8	224+/-18.5	215+/-17.8

No values were statistically significantly different from control value, p<0.05.

In males, increased mean absolute and relative (to final body weight) liver weights were observed in the 100, 500, 750 and 1000 mg/kg/day groups (Table 2). Test material related increases in mean absolute and relative (to final body weight and to brain weight) liver weights were noted for the 500, 750 and 1000 mg/kg/day group females. There was no clear dose response for these increases. These changes in liver weights correlated with an increased incidence and/or severity of periportal hepatocellular vacuolation observed microscopically in the 100 mg/kg/day group males and in the 500, 750 and 1000 mg/kg/day group males and females, although the changes in the livers of the females was minimal.

Table 2

Absolute and relative weights of rats in range-finding study

Parameter	mg/kg/day 0	100	500	750	1000
			males		
Body Wt.	328+/-35	323+/-37	304+/-29	293+/-39	290+/-24*
Liver	11.1+/-1.8	13.4+/-2.1	12.8+/-2.0	12.3+/-2.0	11.8+/-1.0
Liver/BW	3.36+/-0.25	4.12+/-0.26**	4.17+/-0.28**	4.19+/-0.18**	4.08+/0.25**
			females		
Body Wt.	213+/-13	208+/-18	208+/-18	207+/-18	201+/-16
Liver	7.9+/-0.7	8.4+/-0.81	9.5+/-1.1**	9.4+/-0.9**	9.3+/-1.2**
Liver/BW	3.73+/-0.36	4.06+/-0.15	4.58+/-0.32**	4.56+/-0.35**	4.64+/-0.37**

Parameters expressed as means +/- S.D. in grams and due to space limitations have been rounded off.
** Statistically significantly different from control value, p<0.01.

The Lowest-Observed-Effect-Level was 100 mg/kg/day, the lowest dose studied. Thus, a No-Observed-Effect-Level was not established in this study.

Remark : Although the report states that the hepatocellular vacuolization was minimal in females, both control and treated female rats exhibited effects ranging from mild to minimal in severity. In males, the effect was mild at all dose levels, except control, where 1 of 10 exhibited an effect.

Reliability : (1) valid without restriction
1d: Meets generally accepted scientific standards and is described in sufficient detail

08.12.2005 (20)

Type : Sub-chronic
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : 90 day
Frequency of treatm. : daily
Post exposure period : 28 day
Doses : 0, 5, 50 and 500 mg/kg/day based on epoxy equivalent factor of 92.5%
Control group : yes, concurrent vehicle
NOAEL : = 5 mg/kg bw

LOAEL	:	= 50 mg/kg bw
Method	:	OECD Guide-line 408 "Subchronic Oral Toxicity - Rodent: 90-day Study"
Year	:	2001
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : Beginning at 6 weeks of age, groups of Sprague Dawley rats were orally gavaged with 0, 5, 50 or 500 mg/kg/day of ERL-4221. The control and high dose consisted of 25 males and 25 females in each group while the intermediate doses consisted of 20 males and 20 females in each group. Doses were based on an epoxy correction factor of 92.5%. The vehicle was Mazola corn oil. Groups of 15 rats/sex/dose level were assigned to the primary necropsy and groups of 5 rats/sex/dose level from the intermediate dose groups or 10 rats/sex/dose level from the control and top dose group was assigned to a 28-day recovery period. Parameters evaluated included clinical observations, body weights, feed consumption, clinical pathology (hematology, serum chemistry and urinalysis), ophthalmology, vaginal cytology and spermatogenic endpoints. Complete necropsies were performed on all animals and selected organs were weighed. All of the organs specified in the guideline were weighed. Selected tissues were examined microscopically at the primary necropsy as well as the 28-day recovery period. For the control and 500 mg/kg/day groups sacrificed at the primary necropsy, a complete microscopic examination was conducted as per the guideline. For the lower dose levels, microscopic examination was conducted on the liver, kidneys, lung, nasal tissues and any gross lesions noted at the primary necropsy. For the recovery group animals microscopic examination was limited to the liver in the control, 50 and 500 mg/kg/day groups and nasal tissues in the control, 5, 50 and 500 mg/kg/day groups.

All analyses were conducted using two-tailed tests for minimum significance levels of 1% and 5% comparing the control group to the treatment group (by sex). All means were presented with standard deviations (S.D.) and the numbers of sampling units (N) used to calculate the means. Body weight, body weight change, feed consumption, clinical pathology data, estrous cycle data, organ weight data, epididymal and testicular sperm numbers and sperm production rates were subjected to a one-way analysis of variance (ANOVA), followed by Dunnett's test if the ANOVA revealed statistical significance.

The percentage of motile spermatozoa and percentage of sperm with normal morphology were analyzed by the Kruskal-Wallis nonparametric ANOVA test to determine intergroup differences, follwed by the Mann-Whitney U-Test comparing the control and test article-treated groups if the ANOVA revealed statistical significance ($p<0.05$).

Result : All animals survived to the scheduled sacrifice periods. Increased salivation and yellow material on the fur were observed primarily after dosing the 500 mg/kg/day males and females. The yellow material covered the urogential area, and occasionally extended to the neck region. During clinical observations of the high dose female rats which occurred daily after dosing, yellow material was observed a total of 43 times during the 90 day study. Yellow staining of the fur is commonly observed in animals that inadequately groom themselves.

Body weights of the 500 mg/kg/day males were decreased, although not statistically significant, throughout the study. Feed consumption was unaffected in male or female rats receiving up to 500 mg/kg/day. Hematological parameters also appeared to be unaffected in male or female rats receiving up to 500 mg/kg/day. There were no test material-related changes in estrous cycle or spermatogenic endpoints.

At the end of the 13-week dosing period, effects were noted in the kidney,

liver and olfactory epithelium of the nasal tissues. Slight effects in absolute and relative kidney weights in the 500 mg/kg/day group and correlating serum chemistry (increased serum urea nitrogen and phosphorous) and urinalysis (decreased pH and urine creatinine levels (males only)) changes in the 50 and 500 mg/kg/day groups were observed. There were no histopathologic changes noted in the kidneys.

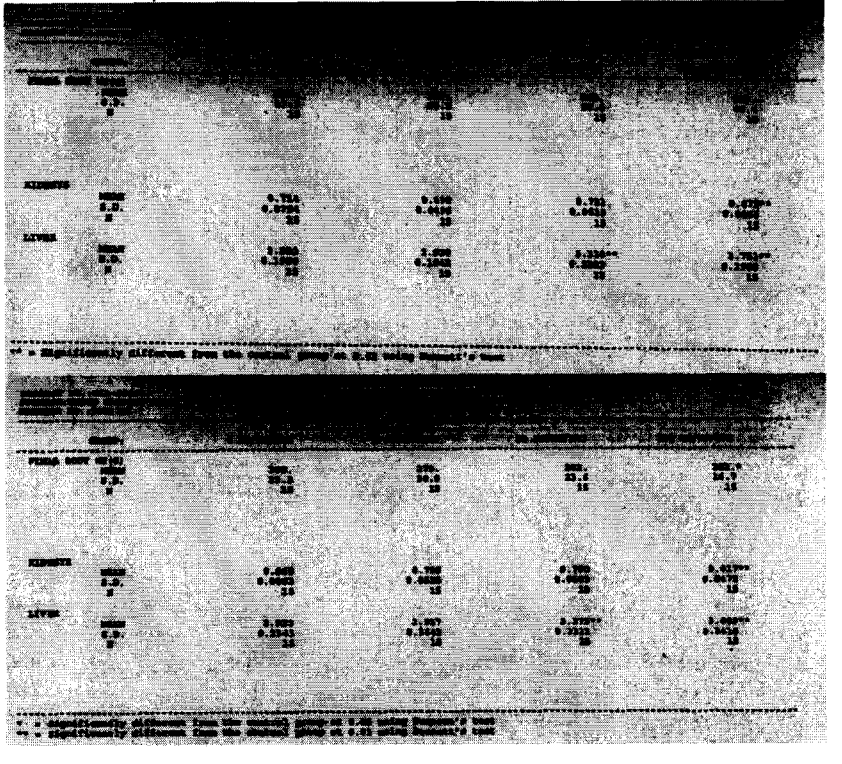
Slight effects were noted in absolute and relative liver weights in the 50 and 500 mg/kg/day groups. Slight changes in liver function, as indicated by serum chemistry alterations (reduced cholesterol levels and increased direct bilirubin and sorbitol dehydrogenase levels), were observed in the 50 and 500 mg/kg/day groups. These effects were accompanied by minimal to mild histopathologic changes in the liver.

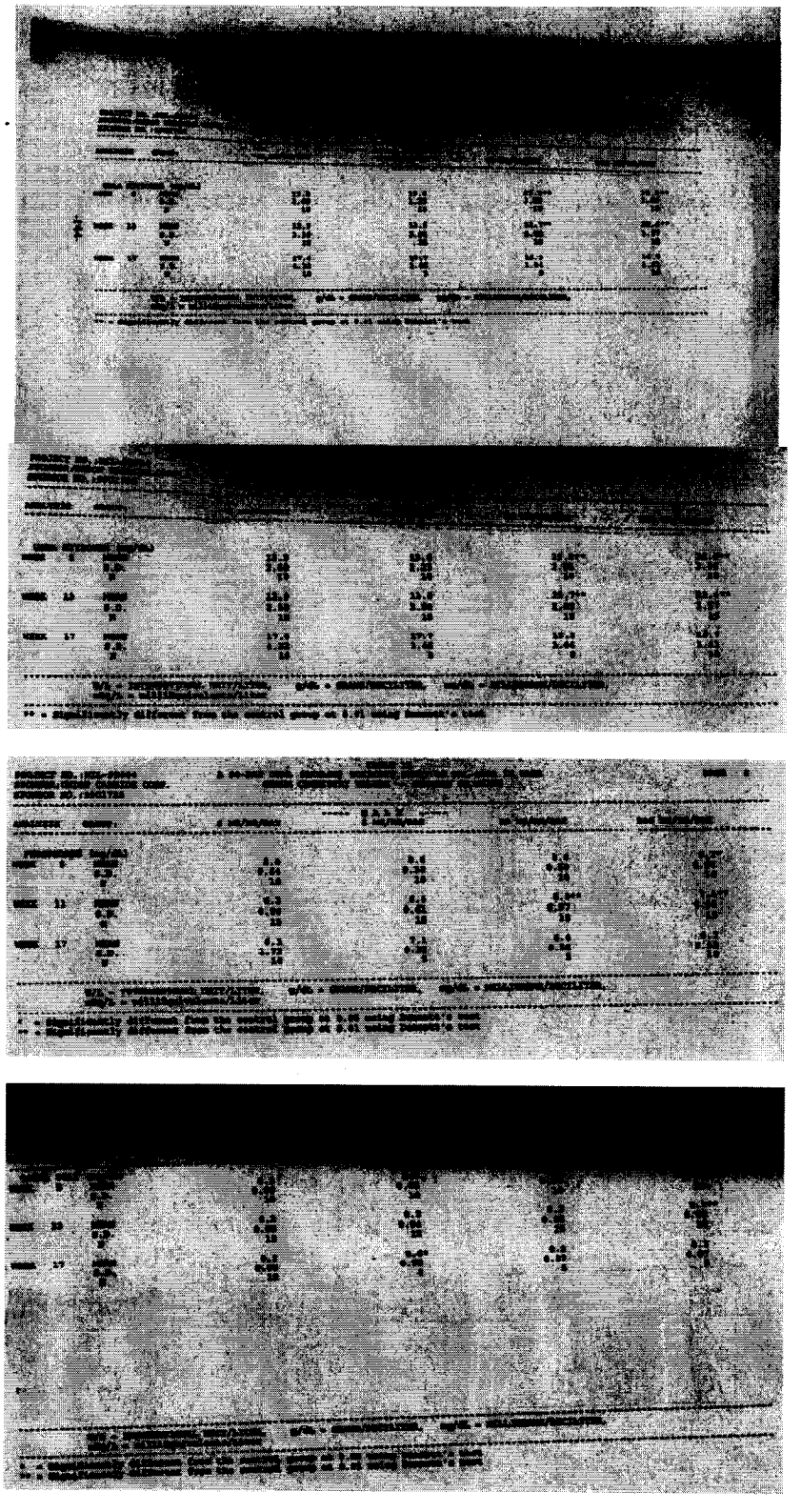
Degeneration of the olfactory epithelium in the nasal tissues was seen in the 50 and 500 mg/kg/day group males and females but not in any of the control group animals.

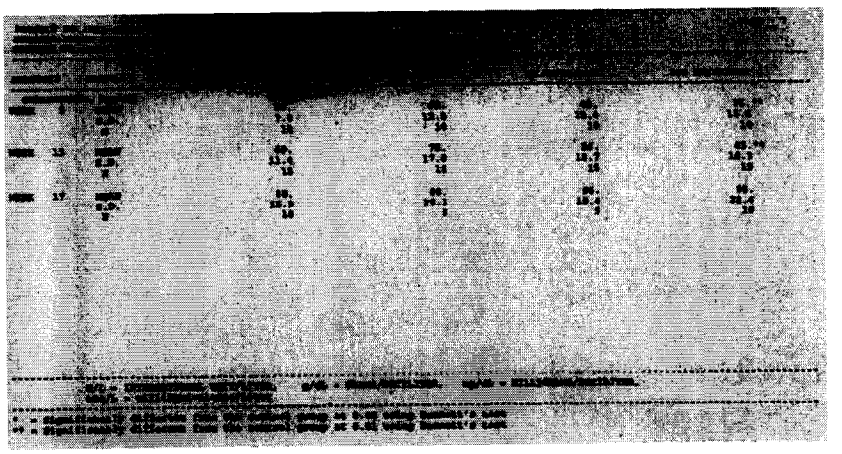
Following a 28-day recovery period, mean body weight of the males from the 500 mg/kg/day dose group remained lower than the controls although weight gain during the period was similar. Olfactory epithelial degeneration was observed in both males and females from the 50 and 500 mg/kg/day groups, although at a lower incidence. In addition, regenerative changes were also evident in the olfactory epithelium.

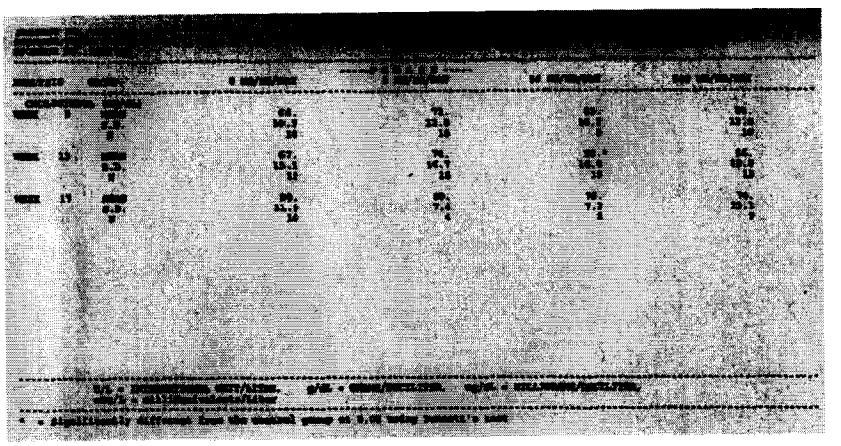
In conclusion, based on the results of this study, the no-observed-effect-level (NOEL) for oral administration of ERL-4221 to rats for a minimum of 90 days was 5 mg/kg/day for both males and females. Both males and females from the 50 mg/kg/day group showed evidence of recovery from all effects following a four-week recovery period; however, lesions in the nasal tissues persisted.

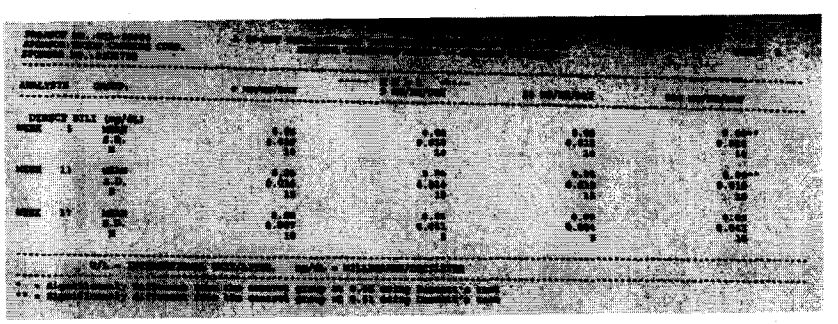
Attached document :

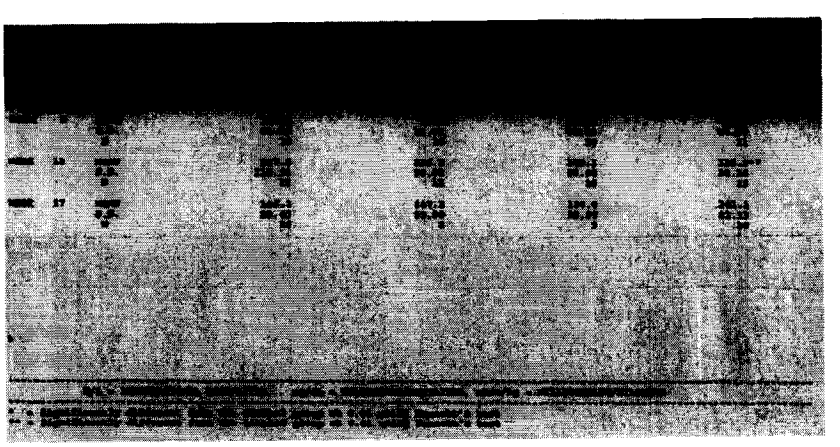


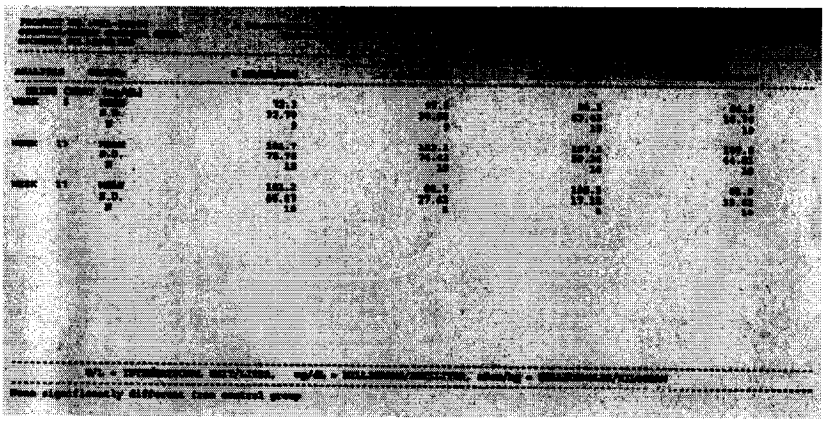


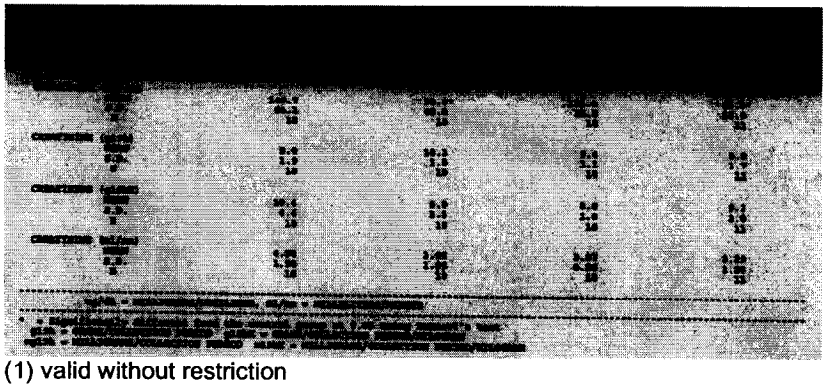












Reliability : (1) valid without restriction
1a: GLP guideline study

08.12.2005 (21)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Ames test
System of testing	:	
Test concentration	:	
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	positive
Method	:	other: essentially follows OECD 471
Year	:	1985
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : For the preliminary toxicity screen, dose levels of 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30 and 117.5 mg/plate were used with strain Salmonella typhimurium strain TA100 only.

For the definitive study, dose levels of 0.1, 0.3, 1, 3 and 10 mg/plate were run in triplicate for each strain of bacteria used. Strains TA98, TA100, TA1535, TA1537 and TA1538 were used. Concurrent solvent, dimethylsulfoxide, and positive controls were run with each test. Both activation-dependent and activation-independent positive controls were used. The activation-independent controls were 4-nitro-o-phenylenediamine for TA98 and TA1538, sodium azide for TA100 and TA1535, and 9-aminoacridine for TA1537. The activation-dependent control in 2-aminoanthracene (2-anthramine) for all strains.

For metabolic activation, samples of Aroclor-1254 induced, rat liver

homogenate (S9) was prepared

Result : In the preliminary screen, complete inhibition of the background lawn growth was observed at doses of 10, 30 and 117.5 mg/plate. Based on these results, mutagenicity testing for the definitive study was conducted at 0.1, 0.3, 1.0, 3.0 and 10 mg/plate.

For the definitive study toxicity was noted at the highest dose, 10 mg/plate, in each strain with and without metabolic activation (Table 1). The only increase in mutagenic activity was observed in strains TA100 and TA1535 with metabolic activation. There was no increase in mutagenic activity in strains TA98, TA1537 or TA1538 with metabolic activation and in any strain without metabolic activation.

Table 1
Results in Ames test with various strains

Strain	Dose, mg/plate	Activation without	with
TA98	Solvent	26+/-3.6	21+/-1.2
	0.1	28+/-6.5	20+/-1.5
	0.3	22+/-4.5	18+/-2.1
	1	24+/-2.6	29+/-1.5
	3	20+/-3.5	27+/-10.1
	10	Toxic	Toxic
Positive Control		885+/-67.0	1490+/-280.9
TA100	Solvent	134+/-4.5	100+/-13.0
	0.1	133+/-8.2	100+/-8.3
	0.3	131+/-6.7	114+/-10.4
	1	135+/-8.5	140+/-7.2
	3	132+/-9.3	253+/-8.7
	10	Toxic	Toxic
Positive Control		2210+/-117.2	1223+/-42.8
TA1535	Solvent	35+/-9.6	9+/-2.1
	0.1	45+/-10.7	14+/-4.7
	0.3	40+/-2.5	18+/-8.5
	1	39+/-12.9	51+/-4.7
	3	39+/-6.1	138+/-15.1
	10	Toxic	Toxic
Positive Control		2256+/-147.0	75+/-10.2
TA1537	Solvent	5+/-1.7	6+/-1.5
	0.1	5+/-1.5	5+/-2.5
	0.3	5+/-1.5	3+/-0.6
	1	7+/-3.1	3+/-2.0
	3	6+/-4.2	8+/-2.6
	10	Toxic	Toxic
Positive Control		91+/-12.1	63+/-27.1
TA1538	Solvent	9+/-1.5	15+/-6.8
	0.1	12+/-4.4	14+/-4.6
	0.3	7+/-1.2	7+/-2.3
	1	5+/-1.0	16+/-3.1
	3	7+/-2.1	14+/-1.0
	10	Toxic	Toxic
Positive Control		1032+/-22.5	206+/-21.1

Reliability : (2) valid with restrictions
2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment

Type	:	Ames test
System of testing	:	preincubation method
Test concentration	:	156 - 5000 ug/plate
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	positive
Method	:	Guidelines for screening mutagenicity testing of chemicals, JAPAN
Year	:	1988
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : Salmonella typhimurium TA98, TA100, TA1535, TA1537 and E coli strain WP2 uvrA- were used at dose levels of 156 to 5000 ug/plate of UVR-6110. The preincubation method was used. Dimethylsulfoxide (DMSO) was used as the vehicle.

Mean number of revertants were calculated for each strain and dose level.

Criteria used for judging the results were as follows: The test substance is positive in this assay when the mean number of revertants is more than double the negative control value and follows a dose-response relationship.

Positive controls used were 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, AF-2
sodium azide, NaN3
9-aminoacridine, 9-AA
N-ethyl-N'-nitro-N-nitrosoguanidine, ENNG
2-aminoanthracene, 2-AA

The S9 mix was prepared from liver homogenate of Sprague-Dawley rats which had received ip injection of phenobarbital and 5,6-benzoflavone.

Result : There was no effect on any strains without metabolic activation (Table 1) and on strains TA98, TA1537 and WP2 uvrA- with metabolic activation (Table 2). The number of revertants induced by the test substance were more than double of the solvent control in strains TA100 and TA1535 with metabolic activation. Microbial growth inhibition was observed, typically at the highest dose. Undissolved test substance was observed on the agar plate at 5000 ug/plate.

Table 1
Results of Ames test without metabolic activation

Strain	Dose/plate (mg)	Average
TA98	Solvent	12
	0.156	12
	0.313	12
	0.625	15
	1.25	15
	2.50	21
	5.00	Toxic
	Positive Control	616
TA100	Solvent	112
	0.156	115
	0.313	133
	0.625	127
	1.25	107
	2.50	49-Toxic
	5.00	Toxic
	Positive Control	507
TA1535	Solvent	11

	0.156	10
	0.313	10
	0.625	15
	1.25	12
	2.50	9
	5.00	Toxic
	Positive Control	216
TA1537	Solvent	4
	0.156	2
	0.313	3
	0.625	4
	1.25	6
	2.50	3
	5.00	Toxic
	Positive Control	796
WP2	Solvent	34
	0.156	33
	0.313	30
	0.625	35
	1.25	38
	2.50	29
	5.00	Toxic
	Positive Control	969

Positive control was 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide at 100 mg for strain TA98 and 10 mg for strain TA100, sodium azide at 500 mg for strain TA1535, 9-aminoacridine at 0.08 mg for strain TA1537 and N-ethyl-N-nitro-N-nitrosoguanidine at 0.002 mg for WP2
No standard deviation provided in report.

Table 2
Results of Ames test with metabolic activation

Strain	Dose/plate (mg)	Average
TA98	Solvent	19
	0.156	23
	0.313	18
	0.625	25
	1.25	23
	2.50	23
	5.00	9-Toxic
	Positive Control	214
TA100	Solvent	131
	0.156	149
	0.313	145
	0.625	157
	1.25	193
	2.50	288
	5.00	Toxic
	Positive Control	815
TA1535	Solvent	17
	0.156	23
	0.313	31
	0.625	43
	1.25	101
	2.50	189
	5.00	Toxic
	Positive Control	79

TA1537	Solvent	9
	0.156	5
	0.313	5
	0.625	8
	1.25	7
	2.50	7
	5.00	1-Toxic
	Positive Control	125
WP2	Solvent	35
	0.156	35
	0.313	31
	0.625	30
	1.25	34
	2.50	40
	5.00	15-Toxic
	Positive Control	947

Positive Control was 2-aminoanthracene at 0.0005 mg for strain TA98, 0.001 mg for strain TA100, 0.002 for strains TA1535 and TA1537 and 0.001 mg for strain WP2.
No standard deviation provided in report.

Reliability : (1) valid without restriction
1a: GLP guideline study

08.12.2005 (23)

Type : HGPRT assay
System of testing : Chinese Hamster ovary cells
Test concentration :
Cycotoxic concentr. :
Metabolic activation :
Result : negative
Method :
Year : 1980
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Method : In the first study, Chinese Hamster ovary cells were exposed for 16 hours to five concentrations of epoxy resin ERL-4221 without the addition of S9 metabolic activation system and for 5 hours to an identical range of concentrations with S9 activation. The 5 concentrations examined were 0.000625, 0.00125, 0.0025, 0.0050 and 0.001%, v/v. The cells used for 5 hours with S9 activation were not assessed for mutant induction because the CO2 concentration in the incubator used for these plates was abnormally high (due to a malfunction) and this malfunction inhibited or killed the cells. In addition, 2 controls were used. A solvent control using DMSO at 20 ul/ml and a H2O control at 20 ul/ml were used for the 3 controls. Positive controls, ethylmethanesulfonate (EMS) at 200 ug/ml without metabolic activation and dimethylnitrosamine at 740 and 3700 ug/ml with metabolic activation, were used also. The surviving fraction was determined at 20 to 24 hours after treatment and the mutant fraction was determined after a 7- to 9-day period to allow the mutant phenotype to express itself. Only the top five concentrations which allowed sufficient cell survival were assessed for survival and induction of mutants. Analysis of mutation frequencies in the CHO test followed the procedure of Irr and Snee which transforms the data prior to parametric analysis using s Student's t test.

The study was repeated with and without S9 activation. Without S9 activation, identical concentrations were used. With S9 activation, concentrations of 0.00125, 0.0025, 0.0050, 0.001 and 0.002%. All other parameters were the same as in the first study.

Result : ERL-4221 was not active in stimulating a dose-related increase of mutant cells when tested either with or without the presence of an S9 metabolic activation system (Table 1). Neither of two experiments provided any indication of a statistically significant mutagenic effect of the test agent. ERL-4221 was considered inactive as an agent for inducing mutation of CHO cells in culture.

Table 1

Chinese Hamster Ovary mutation assay results

Test Chemical	Conc	Total # Viable fraction	Mutant Colonies /10(6)	Mutants viable cells
Experiment 1 without S9				
ERL-4221	0.000625%	0.507	0	0
	0.00125%	0.480	2	4.2
	0.0025%	0.480	0	0
	0.0050%	0.273	1	3.7
	0.0100%	0.163	0	0
DNSO	20 ul/ml	0.900	1	1.1
H2O	20 ul/ml	0.677	1	1.5
medium	-	0.850	0	0
EMS	200 ul/ml	0.600	22	36.7
Experiment 2 without S9				
ERL-4221	0.000625%	0.465	3	6.5
	0.00125%	0.430	2	4.7
	0.0025%	0.342	0	0
	0.0050%	0.218	3	13.8
	0.0100%	0.190	0	0
DNSO	20 ul/ml	0.528	3	5.7
H2O	20 ul/ml	0.615	2	3.3
EMS	200 ul/ml	0.450	124	275.6***
Experiment 2 with S9				
ERL-4221	0.00125%	0.882	14	15.9
	0.0025%	0.722	3	4.2
	0.0050%	0.782	15	19.2
	0.0100%	0.832	15	18.0
	0.0200%	0.780	2	2.6
DNSO	20 ul/ml	0.905	18	19.9
H2O	20 ul/ml	0.965	5	5.2
DMN	3700 ul/ml	0.495	63	127.3***
DMN	740 ul/ml	0.658	30	45.6*

Significantly different from solvent control value, * p<0.05 *** p<0.001

Reliability : (2) valid with restrictions
2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment

08.12.2005 (24)

Type : Sister chromatid exchange assay
System of testing : Chinese Hamster ovary
Test concentration :
Cycotoxic concentr. :
Metabolic activation :
Result :
Method : other: essentially follows OECD 479 guideline
Year : 1980
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Method : In general follows OECD 479 guideline.

A range-finding study was conducted to determine the maximum dose level which would permit survival of at least 50% of the treated cells was based on the prescreening test for cytotoxicity performed as part of the CHO Mutation test.

Concentrations of ERL-4221 tested in the definitive study ranged from 0.00031225 to 0.0100% (by volume). Concentrations tested were 0.0003125, 0.000625, 0.0012.5, 0.0025, 0.0050 and 0.0100% v/v. Negative controls were DMSO at 5 ul/ml and H2O at 5 ul/ml. Positive control was ethylmethanesulfonate (EMS) at 100 ug/ml. CHO cells were exposed to ERL-4221 or appropriate controls for 5 hours without S9 activation. Indirect mutagenic action, required metabolic activation by liver S9 homogenate, was not studied because a highly significant positive response was obtained without metabolic activation which indicated a direct-acting mechanism for this material. Bromodeoxyuridine (BrdU) required to differentiate between the individual 'sister' chromatids by SCE staining, was present at a concentration of 3 g/ml in the growth medium during treatment and during culture period following exposure. A total of 15 cells/dose level and 5 dose levels without metabolic activation were examined. The number of SCE/cell, mean # of SCE/chromosome and the level of statistical significance of the increases above concurrent solvent control values were determined. Data was analyzed using parametric statistical procedres with Student's t-test.

Remark : At concentrations of 0.00125, 0.0025% and 0.005%, a significant increase in the mean number of SCEs/chromosome was observed. Values increased in a dose-response manner 1.65, 2.2 and 3.0-fold higher than the solvent control. Due to the positive effects observed without metabolic activation, tests with a metabolic activation system were not conducted. However, the test without metabolic activation was repeated three times supposedly at the same dose levels due to cytotoxicity. Thus these results may be suspect due to the previously noted cytotoxicity.

Result : ERL-4221 produced statistically significant increases in the SCE frequency at 3 of the 6 dose levels tested in the absence of a metabolic activation system (Table 1). The increase in the numbers of SCE was dose dependent. The test without S9 activation was considered an indication of a significant direct mutagenic action of ERL-4221.

Table 1

Chinese Hamster Ovary Sister Chromatid Exchange results

Test Chemical	Conc	SCE /Cell	Mean # SCE/Chromosome
Without S9			
ERL-4221	0.0003125%	15.53	0.779+/-0.238
	0.000625%	14.67	0.743+/-0.233
	0.00125%	21.07	1.041+/-0.316***
	0.0025%	27.67	1.379+/-0.184***
	0.0050%	38.13	1.869+/-0.301***
	0.0100%	11.60	0.626+/-0.212
DNSO	5 ul/ml	12.13	0.628+/-0.214
H2O	5 ul/ml	13.33	0.680+/-0.169
EMS	100 ul/ml	28.20	1.417+/-0.242***

*** Statistically significant above solvent control, p<0.001

Reliability : (2) valid with restrictions
2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment

08.12.2005 (24)

Type	:	Unscheduled DNA synthesis
System of testing	:	Rat liver cells
Test concentration	:	
Cycotoxic concentr.	:	
Metabolic activation	:	
Result	:	ambiguous
Method	:	other: essentially follows OECD 482 guideline
Year	:	1980
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Method : UDS assay was conducted at concentrations of 0.0001, 0.0010, 0.0030, 0.0100, 0.0300 and 0.1% v/v ERL-4221. The negative solvent control was conducted at 3.0%. Positve controls were 4-nitroquinoline oxide (4-NQO) and DMN. Positive controls were studied at 3-6 concentrations.

Result : In hepatocytes treated with ERL-4221, two of the six concentrations, 0.0010 and 0.0001% tested for potential activity induced a statistically significant increase in the amounts of 3H-thymidine incorporation in the DNA (Table 1). Although there was no indication of a dose-response relationships due to treatment with the test material, the UDS values were sufficiently elevated to suggest a very weak level of mutagenic activity. These data were considered equivocal but suggestive of a questionable-to-weak activity for ERL-4221.

Table 1

Results of unscheduled DNA synthesis in rat hepatocyte

		Radioactivity in DNA	% of solvent Control
Test Chemical	Conc	Mean+/-S.D.	Mean+/-S.D.
ERL-4221	0.0001%	14629+/-993	171.4+/-11.6%**
	0.0010%	12172+/-242	142.6+/-2.8%
	0.0010%	14760+/-1453	172.9+/-17.0%**
	0.0100%	10882+/-1198	127.5+/-14.0%
	0.0300%	7864+/-307	92.1+/-3.6%
	0.1000%	495+/-289	5.8+/-3.4%
DNSO	3.0%	8537+/-3379	100.0+/-39.6%
4-NQO	0.3 ug/ml	10219+/-757	119.7+/-8.9%
	1.0 ug/ml	12011+/-3654	140.7+/-42.8%
	3.0 ug/ml	16720+/-940	195.0+/-11.0%***
DMN	1 ug/ml	16243+/-1690	190.3+/-19.8%***
	10 ug/ml	12198+/-3117	142.9+/-36.5%
	30 ug/ml	13322+/-4554	156.0+/-53.3%**
	100 ug/ml	13195+/-681	154.6+/-8.0%**
	300 ug/ml	14608+/-1669	171.1+/-19.5%**
	1000 ug/ml	9818+/-786	115.0+/-9.2%

Statistically significant above solvent control, ** 0.05>p>0.01 *** 0.01>p>0.001

Reliability : (2) valid with restrictions
2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment

08.12.2005 (24)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	Micronucleus assay
Species	:	mouse
Sex	:	male/female

Strain	:	Swiss
Route of admin.	:	i.p.
Exposure period	:	
Doses	:	500, 1000 and 2250 mg/kg
Result	:	negative
Method	:	OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"
Year	:	1991
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : Probe study - Groups of 3 male and 3 female mice were dosed ip with 500, 1000, 1250, 1500, 1750, 2000, 2250, 2500 and 4000 mg/kg ERL-4221. The test material was dissolved in peanut oil. Mortality and clinical observations were observed during the first day after dosing.

Definitive study - Groups of 18 male and 18 female mice received 0, 500, 1000 and 2250 mg/kg ip. Additional groups of 5 male and 5 female mice received 25 and 40 mg/kg cyclophosphamide in isotonic saline. The same vehicle was used as for the probe study. Bone marrow smears, of 5 animals/dose group, were made at approximately 24, 48 and 72 hours after treatment. For the positive control groups, bone marrow smears were only made at 24 hours. Slides were fixed in methanol and stained with 5% Giemsa for approximately 20 minutes. The percentage of polychromatic erythrocytes (PCE) was counted for a minimum of 1000 erythrocytes.

One-tailed Fisher exact tests and binomial approximation tests were performed to determine if there was a statistically significant increase in the frequency of micronucleated cells in the treated groups. Since multiple comparisons were made, Bonferroni corrections were made to adjust the probability value required for significance. If no difference was detected at the 5% level of significance, the results were negative. If a difference was detected, the dose response was analyzed using the one-tailed Cochran-Armitage test for trend in binomial proportions. If this test detected a trend at the 5% level, the results were cosidered to be positive. If a trend was not detected, the Study Director evaluated the variability observed in the vehicle control and the nature of the statistically significant responses.

Result : Probe study - Mortality was observed within 24 hours of dosing in the 2500 and 4000 mg/kg groups. In addition, decreased motor activity, ataxia, collapse and labored breathing was observed at these dose levels and also at 2250 mg/kg. Based on these results, dose levels of 500, 1000 and 2250 mg/kg were selected for the definitive study.

Definitive study - Clinical signs of toxicity were observed in animals receiving ip doses of 2250 mg/kg. Clinical signs noted were decreased motor activity, collapse, weakness, ataxia and labored breathing.

Cytotoxicity was observed in low and high dose females. Only the increase observed in males treated with 1000 mg/kg and sampled at 48 hours was statistically significant when analyzed with the Fisher exact or binomial approximation test with the Bonferroni correction for multiple comparisons. This increase was not dose-responsive when analyzed with the Cochran-Armitage test for trend in binomial proportions. It is likely that the significance of that response is due to the unusually low spontaneous rate in the control group for that sampling time; therefore the response is not considered to be biologically significant.

Table 1

Frequency of micronucleated polychromatic erythrocytes (MN-PCE)/1000 PCE in bone marrow of mice treated with ERL-4221

	MN-PCE/1000 PCE		
Treatment group	24 hours	48 hours	72 hours
Males			
Control	1.2	0.0	0.4
500 mg/kg	0.8	1.0	0.8
1000 mg/kg	1.0	1.4	0.8
2250 mg/kg	2.0	0.6	0.6
Cyclophosphamide			
25 mg/kg	9.8*		
40 mg/kg	14.2*		
Females			
Control	0.4	0.2	0.2
500 mg/kg	1.8	0.2	1.2
1000 mg/kg	0.6	0.6	0.6
2250 mg/kg	0.8	0.2	1.4
Cyclophosphamide			
25 mg/kg	11.0*		
40 mg/kg	16.2*		

1000 PCE scored/animal, 5 animals scored/dose level

Table 2

Frequency of micronucleated polychromatic erythrocytes (MN-PCE)/5000 PCE in bone marrow of mice treated with ERL-4221

	MN-PCE/5000 PCE		
Treatment group	24 hours	48 hours	72 hours
Males			
Control	6	0	2
500 mg/kg	4	5	4
1000 mg/kg	5	7	4
2250 mg/kg	10	3	3
Cyclophosphamide			
25 mg/kg	49*		
40 mg/kg	71*		
Females			
Control	2	1	1
500 mg/kg	9	1	6
1000 mg/kg	3	3	3
2250 mg/kg	4	1	7
Cyclophosphamide			
25 mg/kg	55*		
40 mg/kg	81*		

5000 PCE scored/animal, 5 animals scored/dose level

Reliability : (1) valid without restriction
1a: GLP guideline study

08.12.2005 (25)

Type : Unscheduled DNA synthesis
Species : rat
Sex : male
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period :
Doses : 0, 500, 1000 and 2000 mg/kg
Result : negative
Method : other: OECD 486
Year : 1999

GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : For the range-finding study, groups of 5 male Sprague Dawley rats were dosed with 1000, 2000, 4000 or 5000 mg/kg via oral gavage. The animals were observed immediately after dosing and daily thereafter for 3 days. Body weights were recorded prior to dose administration and on days 1 and 3 post-dosing.

For the definitive UDS assay, groups of 10 male rats were dosed with 0, 500, 1000 or 2000 mg/kg ERL-4221. Water was used as the vehicle for ERL-4221. A positive control group of the same size received 35 mg/kg dimethylnitrosamine (DMN). Of the ten rats in each group, 3 animals were sacrificed at each time point for hepatocyte culture. Time points were after 2-4 hours and 12-16 hours.

Any mean net nuclear count which was increased by at least 5 counts over the negative control was considered significant. The test article was judged positive if it induced a dose-related increase with no less than one dose significantly elevated above the negative control.

Result : For the range-finding study, all 5 animals died within 3 days of dosing at 4000 and 5000 mg/kg. All animals survived at the two lower dose levels, 1000 and 2000 mg/kg. Clinically, lethargy and piloerection were routinely noted one day after dosing at 2000 mg/kg and greater. All animals appeared to be normal after dosing at 1000 mg/kg.

For the definitive UDS assay, there was no significant increase in the mean number of net nuclear grain counts (i.e., an increase of at least 5 counts over the negative control) after 2-4 hours or 12-16 hours post-dosing (Table 1). The mean number of cells in repair ranged from 4-6% in the controls to 2-3, 2-3 and 2-9 % in the 500, 1000 and 2000 mg/kg groups, respectively, during the two sampling points. The positive control group exhibited 81-99% of the cells undergoing repair. Under these test conditions, ERL-4221 was concluded to be negative in the unscheduled DNA synthesis (UDS) test with mammalian liver cells in vivo.

Table 1
Summary of UDS assay with ERL-4221

Dose Group mg/kg	Time Period	net nuclear grain counts Mean +/- SD
Control	2-4	0.2+/-0.9
500	2-4	0.1+/-0.7
1000	2-4	-0.2+/-0.9
2000	2-4	-0.3+/-0.7
DMN, 35 mg/kg	2-4	17.6+/-1.2*
Control	12-16	-0.2+/-0.4
500	12-16	-0.4+/-0.3
1000	12-16	-0.2+/-0.7
2000	12-16	0.4+/-1.4
DMN, 35 mg/kg	12-16	10.5+/-3.2*

Significantly different from control values.

Reliability : (1) valid without restriction
1a: GLP guideline study

08.12.2005 (26)

Type : other: 32P-postlabeling analysis of in vivo DNA adducts
Species : rat
Sex : male/female

Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	90-day
Doses	:	0 and 500 mg/kg/day
Result	:	negative
Method	:	
Year	:	2000
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4

Method : Samples of liver, kidney and stomach from 5 male rats and liver and kidney from 5 female rats were obtained from the vehicle control and 500 mg/kg/day groups at the time of necropsy in the 90 day study (Padgett, 2001). Groups of 5 male and 5 female rats were treated with mitomycin C as positive controls. No additional information was provided in this report on the positive control group. Tissue samples were frozen in liquid nitrogen until used.

Techniques used to analyze DNA adducts included isolation of DNA from liver, kidney and stomach tissue from male rats and liver and kidney from female rats. Once the DNA was isolated, samples were spectrophotometrically quantified as to purity and amount of DNA. Next, samples of DNA were digested by micrococcal nuclease (MN) and calf spleen phosphodiesterase (SPD) and processed using nuclease P1 as well as butanol enrichment. DNA from mitomycin-C treated animal tissues were analyzed by the nuclease P1 method using TLC Condition 1 only since the adducts of mitomycin-C are adequately purified and identified using these procedures.

The nuclease P1 and butanol enriched DNA digests were 32P-postlabeled with 32P-ATP.

The labeled adducts were chromatographed on anion exchange polyethyleneimine (PEI)-cellulose TLC using two different sets of buffers (TLC Conditions 1 and 2). TLC Condition 1 was used to resolve bulkier base modifications of medium to low polarity. Since it was considered possible that, the test article may have broken down in vivo to smaller components, a different set of buffers (TLC Condition 2) was used to resolve any more polar adducts resulting from the modification of base(s) by smaller molecule(s) derived from the test article. TLC Condition 1 used 1.7M sodium phosphate for approximately 16 hours which was subsequently followed by 1.7M lithium formate and 3.2M urea. TLC Condition 2 used 1.5M formic acid for approximately 16 hours followed by 0.6M ammonium formate.

All the chromatograms from the above TLC developments were dried and scanned using a Packard InstantImager. The chromatograms of control and ERL-4221 treated DNAs were compared to detect any exposure-related new spots. Endogenous spots common to both vehicle control and treated samples were also quantified, when appropriate, to determine if radioactivity of a preexisting spot was affected by treatment.

Remark : The study was conducted in the spirit of compliance with Good Laboratory Practice (GLP) regulations.

Result : Based on the enrichment, labeling and TLC conditions used in this assay, exposure to the highest dose of ERL-4221 (500 mg/kg/day for 90 days) did not induce detectable amounts of new DNA adducts or enhance existing adducts in liver or kidney DNA from male and female Sprague-Dawley rats.

Reliability : (2) valid with restrictions
2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment

08.12.2005 (27)

Type	:	other: 32P-postlabeling analysis of in vivo DNA adducts
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	14 day
Doses	:	0 and 1113 mg/kg/day
Result	:	negative
Method	:	
Year	:	2000
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4

Method :

Samples of liver and kidney from 5 male and 5 female rats were obtained from the vehicle control and 1000 mg/kg/day groups at the time of necropsy in the 14 day study (Padgett, 2000). Groups of 5 male and 5 female rats were treated with mitomycin C as positive controls. No additional information was provided in this report on the positive control group. Tissue samples were frozen in liquid nitrogen until used.

Techniques used to analyze DNA adducts included isolation of DNA from liver and kidney from male and female rats. Once the DNA was isolated, samples were spectrophotometrically quantified as to purity and amount of DNA. Next, samples of DNA were digested by micrococcal nuclease (MN) and calf spleen phosphodiesterase (SPD) and processed using nuclease P1 as well as butanol enrichment. DNA from mitomycin-C treated animal tissues were analyzed by the nuclease P1 method using TLC Condition 1 only since the adducts of mitomycin-C are adequately purified and identified using these procedures.

The nuclease P1 and butanol enriched DNA digests were 32P-postlabeled with 32P-ATP.

The labeled adducts were chromatographed on anion exchange polyethyleneimine (PEI)-cellulose TLC using two different sets of buffers (TLC Conditions 1 and 2). TLC Condition 1 was used to resolve bulkier base modifications of medium to low polarity. Since it was considered possible that, the test article may have broken down in vivo to smaller components, a different set of buffers (TLC Condition 2) was used to resolve any more polar adducts resulting from the modification of base(s) by smaller molecule(s) derived from the test article. TLC Condition 1 used 1.7M sodium phosphate for approximately 16 hours which was subsequently followed by 1.7M lithium formate and 3.2M urea. TLC Condition 2 used 1.5M formic acid for approximately 16 hours followed by 0.6M ammonium formate.

All the chromatograms from the above TLC developments were dried and scanned using a Packard InstantImager. The chromatograms of control and ERL-4221 treated DNAs were compared to detect any exposure-related new spots. Endogenous spots common to both vehicle control and treated samples were also quantified, when appropriate, to determine if radioactivity of a preexisting spot was affected by treatment.

Result : Based on the enrichment, labeling and TLC conditions used in this assay, exposure to the highest dose of ERL-4221 (1000 mg/kg/day for 14 days) did not induce detectable amounts of new DNA adducts or enhance existing adducts in liver or kidney DNA from male and female Sprague-Dawley rats.

Remark : The study was conducted in the spirit of compliance with Good Laboratory Practice (GLP) regulations.

Reliability : (2) valid with restrictions
2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment

08.12.2005 (28)

5.7 CARCINOGENICITY

Species	:	mouse
Sex	:	male
Strain	:	
Route of admin.	:	dermal
Exposure period	:	3 days/week (Monday, Wednesday and Friday)
Frequency of treatm.	:	
Post exposure period	:	
Doses	:	undiluted
Result	:	negative
Control group	:	other: acetone treated
Method	:	
Year	:	1964
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Method : Groups of 40 C3H/Anf mice were painted on Monday, Wednesday and Friday of each week until they died. The control mice received acetone while the treated mice received undiluted EP-221. The positive control mice received 0.2% methyl cholanthrene dissolved in acetone. The hair of the mice was clipped once/week. The material was applied to the mouse skin with one brushfull from a series 197 #1 Grumbacher brush for each application. Mice receiving EP-221 were painted for 28 months while the vehicle controls were painted with acetone for 26 months and the positive controls were painted with methyl cholanthrene for 13 months.

Result : Survival of EP-221 treated mice was slightly better than vehicle control mice (Table 1). For EP-221, after 23 months of skin painting, only one tumor was observed at the application site.

No additional information provided.

Table 1
Summary of skin painting results

	Vehicle control	Positive control	EP-221
Initial Number of mice	40	40	40
# alive after 12 months	32	2	39
# alive after 18 months	26	0	31
# alive after 24 months	4	0	12
First tumor observed	23	3	23
# of mice with tumors	2	39	1

Remark : A brushfull is approximately 24.8 mg EP-221. Assuming the average weight of the male mouse was 40 grams, this corresponds to 620 mg/kg.

Reliability : (2) valid with restrictions
2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment

08.12.2005 (29)

5.8.1 TOXICITY TO FERTILITY

Type	:	other: 90 day oral gavage study
Species	:	rat

Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	90 day
Frequency of treatm.	:	
Premating exposure period		
Male	:	
Female	:	
Duration of test	:	
No. of generation studies	:	
Doses	:	0, 5, 50 and 500 mg/kg/day based on epoxy equivalent factor of 92.5%
Control group	:	yes, concurrent vehicle
NOAEL parental	:	>= 500 - mg/kg bw
Method	:	other: OECD 408
Year	:	2001
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : Groups of Sprague Dawley rats were orally gavaged with 0, 5, 50 or 500 mg/kg/day of ERL-4221. The control and high dose consisted of 25 males and 25 females in each group while the intermediate doses consisted of 20 males and 20 females in each group. Doses were based on an epoxy correction factor of 92.5%. The vehicle was Mazola corn oil. Groups of 15 rats/sex/dose level were assigned to the primary necropsy and groups of 5 rats/sex/dose level from the intermediate dose groups or 10 rats/sex/dose level from the control and top dose group were assigned to a 28-day recovery period. Parameters evaluated included clinical observations, body weights, feed consumption, clinical pathology (hematology, serum chemistry and urinalysis), ophthalmology, vaginal cytology and spermatogenic endpoints. Vaginal cytological endpoints included vaginal smears beginning 22-23 days prior to the primary necropsy and continued to the day of necropsy. The average cycle length was calculated for complete estrous cycles. Spermatogenic endpoints included as assessment of sperm motility and morphology. At least 200 (if possible) sperm were analyzed for motility and morphology from each animal. Complete necropsies were performed on all animals and selected organs were weighed. These organs included epididymides (total and cauda), testes, uterus (with cervix) and ovaries (with oviducts). Selected tissues were examined microscopically at the primary necropsy as well as the 28-day recovery period. These selected tissues included epididymides, prostate and testes of males and mammary gland, ovaries with oviducts, uterus with cervix and vagina of females. The right testis and epididymis were fixed in Bouin's solution prior to histopathologic examination.

Result : Subchronic toxicity endpoints are discussed in the repeated dose section of this dossier.

There were no test material-related changes in estrous cycle or on spermatogenic endpoints (mean testicular and epididymal sperm numbers, sperm production rate and sperm molity and morphology) observed (Table 1).

Table 1
Results of estrous and spermatogenic endpoints

	Dose Level, mg/kg/day			
Parameter	0	5	50	500
Females				
estrous cycle length, days	4.6+/-0.93	4.7+/-0.91	5.2+/-1.81	4.9+/-1.27
Males				

sperm/gram tissue in left testis, in millions
95.4+/-21.01 110.7+/-32.04 113.6+/-22.35 102.3+/-14.13
sperm/gram tissue in left epididymis, in millions
348.9+/-108.9 340.6+/-79.8 381.8+/-153.46 385.0+/-147.58
sperm motility assessment, %
82.9+/-9.92 83.3+/-7.76 81.0+/-13.5 74.4+/-16.47
sperm morphology
normal, %
99.4+/-0.68 99.8+/-0.32 99.6+/-0.62 98.87+/-1.17
normally shaped head separated from flagellum, %
0.5+/-0.64 0.1+/-0.30 0.4+/-0.61 0.8+/-0.77
head absent with normal flagellum, %
0.0+/-0.13 0.1+/-0.18 0.1+/-0.18 0.3+/-0.56

Mean+/-S.D.
No values were significantly different from control group, $p<0.05$.

There were no treatment-related effects noted on reproductive organ weights of male or female rats gavaged with ERL-4221 for 90 days. Mean absolute prostate weight was slightly decreased in the 5 mg/kg/day group which was not considered to be treatment-related.

There were no treatment-related microscopic findings noted on reproductive organs of male or female rats. All microscopic findings were consistent with normal background lesions in clinically normal rats of the strain and age used in this study and were considered to be spontaneous and/or incidental in nature and unrelated to test material administration.

Reliability : (1) valid without restriction
1a: GLP guideline study

08.12.2005 (21)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	gestation days 6-19
Frequency of treatm.	:	daily
Duration of test	:	
Doses	:	0, 5, 25, 125 and 500 mg/kg/day (based on epoxy equivalent weight)
Control group	:	yes, concurrent vehicle
Method	:	OECD Guide-line 414 "Teratogenicity"
Year	:	2003
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : Groups of 25 bred female rats were orally administered 0, 5, 25, 125 or 500 mg/kg/day based on epoxy equivalent weight of ERL-4221 on gestation days 6-19. These dosages corresponded to 0, 5.4, 27, 135 and 541 mg/kg/day of ERL-4221, respectively. The control group received the vehicle, Mazola corn oil. Clinical observations, body weights and feed consumption were recorded at appropriate intervals. On gestation day 20, all females were euthanized for a scheduled laparohysterectomy. The uteri and ovaries were examined and the number of fetuses, early and late absorptions, total implantations and corpora lutea were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The liver and kidneys of each female were weighed. The fetuses were weighed, sexed, examined for external,

visceral and skeletal malformations and developmental variations. Heads from approximately one-half of the fetuses in each litter were placed in Bouin's fixative for subsequent soft-tissue examination by the Wilson sectioning technique. The heads from the remaining one-half of the fetuses were examined by a mid-coronal slice. All carcasses were eviscerated, fixed in 100% ethyl alcohol and stained with Alizarin Red S and Alcian Blue. External, visceral and skeletal findings were recorded as developmental variations (alterations in anatomic structure that are considered to have no significant biological effect on animal health or body conformity, representing slight deviations from normal) or malformations (those structural anomalies that alter general body conformity, distrupt or interfere with body function, or may be incompatible with life.

Mean maternal body weights (absolute and net), body weight changes (absolute and net) and food consumption, gravid uterine weights, organ weights, number of corpora lutea, implantation sites and viable fetuses, and fetal body weights (separately by sex and combined) were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test was used to compare the test article-treated groups to the control group. Mean litter proportions (percent per litter) of prenatal data (viable and nonviable fetuses, early and late resorptions, total resorptions, pre- and post-implantation loss, and fetal sex distribution) were subjected to the Kruskal-Wallis nonparametric ANOVA test to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, the Mann-Whitney U-test was used to compare the test article-treated groups to the control group. Mean litter proportions (percent per litter) of total fetal malformations and developmental variations (external, visceral, skeletal and combined) and of each particular external, visceral and skeletal malformation or variation were analyzed by the Kruskal-Wallis nonparametric ANOVA test followed by the Mann-Whitney U-test (if appropriate) as described above.

Result : All females survived to the scheduled necropsy. The clinical condition of the animals in the test article-treated groups was not adversely affected by ERL-4221. No test material-related maternal or fetal effects were observed in the 5 and 25 mg/kg/day groups. No test material-related fetal malformations were observed at any dose level; no test material-related developmental variations were noted in the 5, 25 and 125 mg/kg/day groups. Intrauterine growth and survival in the 125 mg/kg/day group were unaffected by test material administration.

Unless specified all values listed below were statistically significantly different from control values.

Test material-related effects noted in the 125 mg/kg/day group consisted of:

- Transient, initial mean body weight losses (for gestation day 6-7 weight gain was 0 and 6 grams for the control and 125 mg/kg/day group, respectively, and was comparable throughout the remainder of gestation) and reduced feed consumption (for gestation day 6-7 food consumption was 73 and 61 g/kg/day for the control and 125 mg/kg/day group, respectively; for gestation day 7-8 food consumption was 76 and 65 g/kg/day for the control and 125 mg/kg/day group (not statistically significant), respectively; gestation days 6-9 average food consumption was 74 and 68 g/kg/day for the control and 125 mg/kg/day groups, respectively and was comparable throughout the remainder of gestation). These observations were not considered to be adverse due to the rapid amelioration of the effects.
- Macroscopic findings in the kidney (one female with depressed areas grossly visible) in conjunction with increased mean kidney weight (absolute kidney weight was 2.09 and 2.29 g in the control and 125 mg/kg/day,

respectively).

Test material-related effects noted in the 500 mg/kg/day group consisted of:
- Mean maternal body weight losses (gestation day 6-7 weight gain was 0 and -11 for the control and 500 mg/kg/day group, respectively) and reduced body weight gain (gestation days 6-9 weight gain was 5 and -2 grams for the control and 500 mg/kg/day group, respectively; for gestation days 6-20 weight gain was 115 and 94 grams in the control and 500 mg/kg/day group, respectively) and food consumption (for gestation day 6-7 food consumption was 73 and 46 g/kg/day for the control and 500 mg/kg/day group, respectively; for gestation day 7-8 food consumption was 76 and 71 g/kg/day for the control and 500 mg/kg/day group, respectively (not statistically significant); for gestation days 6-9 food consumption was 74 and 64 g/kg/day for the control and 500 mg/kg/day group and was comparable throughout the remainder of gestation with the exception of gestation day 19-20; food consumption on gestation day 19-20 was 65 and 55 mg/kg/day for the control and 500 mg/kg/day group, respectively).
- Increased macroscopic findings in the kidney (two females with drepressed area grossly visible) in conjunction with increased mean kidney weight (absolute kidney weight was 2.09 and 2.34 g in the control and 500 mg/kg/day group, respectively).
- Reduced mean fetal body weight (3.6 and 3.3 g for the control and 500 mg/kg/day group, respectively).
- Increased skeletal developmental variations (reduced mean litter proportion of cervical centrum #1 ossified (25.7 and 11.7% in the control and 500 mg/kg/day group, respectively) and increased mean litter proportions of unossified sternebrae (unossified sternebrae nos 5 and/or 6 were 7.6 and 26.4% in the control and 500 mg/kg/day group, respectively; unossified sternebrae nos 1,2,3 and/or 4 were 0.3 and 1.6% in the control and 500 mg/kg/day group, respectively). Although the incidence of unossified sternebrae were not statistically significant, they were above the maximum values in the historical control data and thus were attributed to the test material as further indication of developmental delay.

Based on the results of this study, the no-observed-adverse-effect-level (NOAEL) for maternal toxicity was 25 mg/kg/day following oral administration. For prenatal developmental toxicity the NOAEL was considered to be 125 mg/kg/day.

Reliability : (1) valid without restriction
1a: GLP guideline study

08.12.2005 (30)

Species	:	rat
Sex	:	
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	days 6-19 of gestation
Frequency of treatm.	:	daily
Duration of test	:	
Doses	:	0, 50, 100, 250, 500 and 750 mg/kg (based on epoxy equivalent weight)
Control group	:	yes, concurrent vehicle
Method	:	other: probe study for definitive OECD 414 study
Year	:	2003
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : Groups of eight bred Sprague Dawley rats were dosed via oral gavage on gestation days 6-19. Target dosage levels were 0, 50, 100, 250, 500 and 750 mg/kg/day of epoxy equivalent weighted ERL-4221. These dose levels corresponded to 0, 54, 108, 270, 541 and 811 mg/kg/day of actual ERL-4221, respectively. The control group received the vehicle, Mazola

corn oil. Clinical observations, body weights and feed consumption were recorded. On gestation day 20, all females were euthanized for a scheduled laparohysterectomy. The uteri and ovaries were examined and the number of fetuses, early and late absorptions, total implantations and corpora lutea were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The liver and kidneys of each female were weighed. The fetuses were weighed, sexed, examined for external malformations and developmental variations and discarded.

Result : All maternal animals survived to the scheduled laparohysterectomy on gestation day 20. No test article-related clinical findings were observed.

Mean body weight gains were reduced in the 500 and 750 mg/kg/day groups during gestation days 6-9, 12-20 and when the overall treatment period (gestation day 6-20) was evaluated. Mean body weights (gestation days 11-20), gravide uterine weights, net body weights and net body weight gains in these dose groups were also reduced. Feed consumption in the 500 and 750 mg/kg/day group was slightly reduced during gestation days 6-9 and 12-20. Feed consumption in the 50, 100 and 250 mg/kg/day groups was not affected by the test article.

No test article-related macroscopic changes or effects on liver and kidney weights were noted at the scheduled necropsy on gestation day 20.

Mean fetal weights were reduced in the 500 and 750 mg/kg/day groups.

No external malformations or developmental variations were observed in fetuses at any dose level.

In conclusion, maternal toxicity was expressed at dose levels of 500 and 750 mg/kg/day by effects on body weights and feed consumption. Prenatal developmental toxicity was expressed by reduced fetal body weight at dose levels of 500 and 750 mg/kg/day. No maternal or prenatal developmental toxicity was expressed at dose levels of 50, 100 or 250 mg/kg/day. Based on this data, dose levels of 5, 25, 125 and 500 epoxy equivalent weight were used for the definitive study.

Reliability : (2) valid with restrictions
2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment

08.12.2005 (31)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

8.1 METHODS HANDLING AND STORING

8.2 FIRE GUIDANCE

8.3 EMERGENCY MEASURES

8.4 POSSIB. OF RENDERING SUBST. HARMLESS

8.5 WASTE MANAGEMENT

8.6 SIDE-EFFECTS DETECTION

8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER

8.8 REACTIVITY TOWARDS CONTAINER MATERIAL

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10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT